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UNIVERSITY OF ALBERTA

PHYTOCHEMICAL INVESTIGATION OF *MATRICARIA MARTIMA*

BY

MESFIN BEKELE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

IN

PHARMACEUTICAL SCIENCES (MEDICINAL CHEMISTRY)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

FALL, 1995

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The undersigned certify that they have read, and recommended to the faculty of Graduate Studies and Research for acceptance, a thesis entitled PHYTOCHEMICAL INVESTIGATION OF MATRICARIA MARTIMA submitted by Mesfin Bekele in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN PHARMACEUTICAL SCIENCES (MEDICINAL CHEMISTRY).

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Date: September 18, 1995

TO AYELECH AND HILAWI

ABSTRACT

Matricaria martima is one of the many wild flowering plants growing in Alberta, Canada. This noxious weed is particularly abundant in the Edmonton area. The active constituents of the plant havee not been investigated. *M. martima* belongs to the same family as the chamomiles; therefore a chemical relationship is expected.

In this study chromatographic methods, which revealed the presence of at least fifteen different compounds, were developed for the purpose of detection and separation. The initial stage of separation was carried out by conventional column chromatography in a solvent system of methanol and water. The detection and isolation of the individual components was performed by thin layer chromatography and preparative thin layer chromatography, using ethyl acetate, methanol and water as a solvent.

Among the fifteen detected compounds five of them were isolated. Based on the data obtained from TLC, melting point, UV spectroscopy, mass spectroscopy and NMR spectroscopy, two of the components are characterized as apigenin and scopoletin. The third compound is tentatively identified to be tomentin.

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Faculty of Pharmacy and Pharmaceutical Sciences
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CHAPTER I

INTRODUCTION

1. Research in traditional medicine

1.1 Botanical methods in ethnopharmacology

An imperative demand imposed on all scientific investigations is that they should be repeatable, which calls for adequate documentation. In medicinal plant research, botanical documentation plays a vital role. Without correctly identified material and properly documented voucher specimens the results are at best suspect and at worst useless.

To a botanist, botanical methods in ethnopharmacology are fundamental and should be well known to every one involved in research on medicinal plants. Schultes (1991) indicates that this is not the case, since he defined ethnopharmacology as "the observation, identification, description and experimental investigation of the ingredients and the effects of indigenous drugs". Ethnopharmacology is a highly interdisciplinary field where botany plays a vital role, and to leave out the identification of plants yielding those indigenous drugs is to leave out the very basis for ethnopharmacological research.

A focus on botany in ethnopharmacology is important because this knowledge and methodology is essential for the conservation of medicinal plants. Although conservation is a

very important matter, it is not regarded as a pure science. It is vital for the future of ethnopharmacological research. The threat to many species used for medicinal purposes is very grave and if scientists do not care about conservation, many species might become extinct in the near future.

The evaluation of traditional medicinals varies depending on which part of the world we are dealing with. In some parts of the world e.g., the tropics and China, traditional medicine has always held a strong position. This is not the case in North America or in most parts of Europe. The evolution of modern schools of medicine in those parts meant that traditional medicine, including the use of medicinal plants, was looked upon with disregard or even contempt by the orthodox medical profesion.

The reawakening of the interest in plant substances in the industrialized countries, together with the rapidly growing interest in developing countries to start research programmes in ethnopharmacology, makes it imperative to secure and widen the knowledge about the importance of botany in such research.

The basic role played by botany in research dealing with plant substances is demonstrated in table 1. Botanical expertise is needed not only for identification but also for inventories and documentation. All three are closely interwoven, since inventories are meaningless and safe identification impossible unless proper documentation is

achieved simultaneously.

A program of study begins with laborious field work, by a team which include at least one botanist, one ethnopharmacologist and local traditional healers. The information from those traditional healers is essential since the possibility of finding active substances is much greater if plants used by the healers are investigated, than if plants are sampled at random (Spjut and Perdue, 1978). In order to secure proper documentation, a standardized form should be filled for each collection, giving the scientific name of the plant (if possible), vernacular name, locality, part(s) of the plant used, mode of adminstration, etc.

Table 1. Program for studies of plants in traditional medicine (Samuelsson,1988)

A. Inventory and botanical identification
B. Studies of the literature
C. Pharmacological screening of extracts
D. Isolation and identification of pharmacologically active constituents
E. Pharmacological studies on isolated substances
F. Toxicological studies
G. Clinical testing
H. Production of drugs

After the field work has been completed the most urgent task is the identification of the specimens collected, which again makes the availability of botanists crucial. Whereas

some material may be named without any particular difficulty, identification could in other cases be time-consuming and sometimes very difficult.

The naming of a specimen in the field quite often offers considerable difficulties or is impossible. In such cases the vernacular name may be used as a reference, to be later replaced by the scientific name. It is then extremely important to be aware of the fact that the same vernacular name sometimes is applied to different species. On the other hand, different vernacular names are sometimes used for the same species. If a voucher specimen is not available, this will cause considerable confusion.

As mentioned above, an imperative demand on every scientific investigation is that it should be repeatable, and one of the most difficult problems in research on medicinal plants is that analysis from a second batch of material often gives discordant results compared with the first analysis. This may in some cases be due to variation from one lot to another in the concentration of active substances, but may be more often due to the fact that the second sample was from a different species. Such failures can only be avoided if documenting collections with complete voucher herbarium specimens are secured of all plants investigated and information on these vouchers is published with each report on active substances.

1.2. Anthropological methods in ethnopharmacology

Anthropology's contributions to ethnopharmacology are distinguished less by specific methods than by a critical and biobehavioral perspectives. In anthropological studies medicinal plants are viewed as cultural objects- "human artifacts" or "transacted symbols" (Dow, 1986) and also as biodynamic elements that have infinite pharmacological potential. Both of these aspects reflect people's interpretations and manipulations of their physical and social environment.

The value of ethnographic study has generally been acknowledged only for the first tier of basic research, that is for generating, from field study and literature review information regarding the when, where and how, of plant utilization. The potential contribution of ethnographic study to later phases of investigation has been overlooked: details of culture and behaviour have been considered extraneous to laboratory investigations of pharmacologic mechanisms and clinical evaluations of medicinal plants (Kyerematen, 1987).

Typically, anthropologists do not seek to discover new drugs or otherwise advance biomedicine, although their work may serve that end (Etkin,1993). As Nina L. Etkin (1993) says "the unique contributions of anthropology to ethnopharmacology rest with the depth to which we develop an ethnographic base that can be correlated with laboratory and

clinical investigations in such a way that we comprehend contextually how people's medicinal and other actions affect their health".

In general, anthropological methods in ethnopharmacology are meant to advance a critical and biobehavioral perspective for the contraction of primary data in the light of indigenous paradigms of health and therapeutics. The unique contributions of anthropology are the conceptual and practical tools that allow one to develop the ethnography of plant use in sufficient depth to correlate with laboratory and clinical investigations of plant constituents and activities. This serves an ethnopharmacology that links bioscientific research to traditional empirical knowledge. Specific methods used in anthropological studies include key respondents, participant observations, focus groups, structured and unstructured interviews, survey instruments and questionnaires, lexical and semantic studies, and discourse and content analysis of oral tradition, and archival and other literature review (Etkin, 1993).

1.3. Chemical methods in ethnopharmacology

Ethnopharmacology has as a prerequisite a close collaboration between ethnologists, botanists, pharmacologists and chemists. It is not possible for the researcher to master all of these specialties, but it is important that he/she has a general knowledge of what can and what cannot be done with the various techniques.

Thanks to immensely improved spectroscopic techniques, even very complex structures may now be solved on a milligram scale in as many weeks or months as it took years not long ago and at the expense of grams of substances in degradation experiments.

Thanks to the rapid development of new, "soft" ionization methods and instrumental configurations, the applicability of mass spectrometry (MS) has expanded very much, e.g., for structural analysis of peptides (Wickberg, 1993). The vastly improved functional reliability of recent high resolution instruments makes them suited even for routine determination of molecular formulae on a microgram or lower scale. This can also be done on line in gas chromatography-mass spectrometry (GC-MS) or high performance liquid chromatography-mass spectrometry (HPLC-MS) experiments, and even thin layer chromatography (TLC) plates may be scanned in a high resolution mode with the fast atom bombardment (FAB) technique (Wickberg, 1993).

Fourier transform nuclear magnetic resonance (FTNMR) has created a new dimension in structural organic chemistry within the last decade. Conventional NMR, at say 100 MHz for proton spectra, usually exhibited complex second order coupling patterns and therefore complete assignments could only rarely be made. Now FT NMR instruments with super conducting magnets and operating at up to 600 MHz are commercially available. These high frequency spectrometers have a vastly improved performance with respect to both resolution and sensitivity, and the fast development in computer technology allows even more sophisticated experiments with further gain in speed and information.

Borje Wickberg (1993) has two strong recommendations to scientists entering the field of natural products. Firstly, never launch a major project unless a sufficient supply of starting material is guaranteed, be it a plant, animal or a locally used drug. Secondly, an adequate bioassay should be found or developed for monitoring the isolation of active constituents. The assay should allow quantitative estimates but at the same time be both rapid and sensitive enough so that, for example, series of chromatographic fractions can be tested without great loss of time and substance.

For initial extractions, solvents are usually chosen for their efficiency. However sometimes it is essential to avoid extracting too much and by choosing, for example, aqueous rather than anhydrous methanol for a nonpolar

constituent, interfering contaminants may be eliminated from the very beginning.

It is rare to have a 100% recovery upon routine chromatography on adsorbents like alumina and silica. The reasonably stable fungal sesquiterpene isovelleral can be taken as a drastic example. Upon flash chromatography on alumina it undergoes a specific oxidation with approximately 50% conversion (Sterner, 1988), while quick filtration through deactivated alumina is useful for removing fatty acids in the isolation of isovelleral.

Organic adsorbents such as Amberlite XAD-2 resin or Sephadex LH20 (gel filtration) are devoid of catalytic properties and are commonly used particularly in early stages of separation schemes. However, desire to avoid the negative effects of adsorbents has stimulated the interest in liquid-liquid partitioning methods ranging from simple extraction in separatory funnels to sophisticated continuous techniques collectively known as counter-current chromatography or CCC (Hustettmann, 1986; Foucault, 1991). A common feature is that the mobile phase is pumped slowly through the stationary phase so that continuous phase mixing and separation occurs.

1.4. Pharmacological methods in ethnopharmacology

Ethnomedical treatments are used to treat most disease states and medical conditions known to modern medicine. Ethnopharmacological evaluation of the efficacy of these ethnomedical treatments rely upon a large number of different pharmacological models. A discussion of the models in use would require model evaluation for all the major organ systems including the nervous system (central, peripheral and autonomic), the cardiovascular system, renal system, reproductive system, hormone system, and a variety of infectious disease models for viral, fungal, parasitic and bacterial infections. Many pharmacological models are also defined in terms of disease states such as inflammation and cancer.

The primary goals of ethnopharmacological investigations are to evaluate and verify pharmacological activity of an ethnomedical treatment. The verification of an ethnomedical treatment for efficacy, and encouraging its use as a remedy is an important goal often forgotten or neglected by modern scientists. Clearly, some ethnomedical preparations are simple placebos. Other preparations are without any actions and are used to treat self-limiting diseases such as minor influenza or simple cold. Identifying and characterizing the efficacious ethnomedical treatments for a variety of significant disease states could generally benefit indigenous populations in several ways, and more

efforts should be focused on these goals (Waller, 1993).

Large numbers of people in developing countries have poor access to modern health care systems and the associated drugs used in the treatment of diseases. Most countries with frequent usage of ethnomedical treatments have many traditional practitioners preparing ethnomedicines or providing preparation instructions to local populations. These practitioners could be used to great advantage if they were organized and encouraged to use only efficacious and safe ethnomedicines while discouraging the use of ineffective and potentially toxic ethnomedicines. Such activities could be supported by performing scientific evaluations of efficacy for local ethnomedical preparations as well as organizing and disseminating scientific information to the local traditional practitioners. Limited availability of modern drugs is primarily due to the cost of modern drugs to already impoverished governments. The components used in ethnomedicine, however, are relatively inexpensive and accessible since most ethnomedicines are formulated from locally grown and produced plant products. Most importantly, the cost is usually appropriate for the economic situation of the local area. The availability of a local pharmacopoeia supported by scientific data could positively impact on the health status of large populations without access to modern drugs.

A good scientific approach to ethnopharmacological investigations requires the use of information from several disciplines. Ethnopharmacological, ethnobotanical, phytochemical and toxicological information must be obtained and evaluated along with the ethnopharmacological data.

The pharmacological evaluations are often not adequately justified using ethnomedical information. The ethnomedical information is either poorly presented or absent in the manuscripts. The selection of a pharmacological method to evaluate an activity, without good ethnopharmacological justification, is the same as randomly screening plant materials for pharmacological activity. Complete ethnomedical information is essential (Waller, 1993).

CHAPTER II

LITERATURE REVIEW

2.1. Botany

2.1.1. Botanical source

The common name chamomile usually refers to either of the following two species: Roman or English chamomile, *Chamaemelum nobile* (L) ALL. syn. *Anthemis nobilis* L.; German or Hungarian chamomile, *Chamomilla recutita* (L) Rausch. syn. *Matricaria chamomilla L.*

Other related species include: Scentless or wild chamomile, *Matricaria martima* L. Var. agrestis (Knaf.) Wimott; Pineapple weed, *Matricaria matricaroides* (Less.) Porter; Stinking mayweed, *Anthemis cotula* L.

All species belong to the family Compositae (Astereaceae).

The name chamomile is taken from the Greek words "Kamai" (on the ground) and "Melon" (an apple) or ground-apple and refers to Roman chamomile being a low growing plant with a distinct aromatic scent of apple (Grieve, 1982; Marylin, 1977; Hamon, 1989; Moss, 1959)

2.1.2. Botanical description

2.1.2.1. German chamomile

German chamomile, also known as Hungarian, Single or Wild chamomile is *Matricaria chamomilla*. This plant has a

distinct, apple-like fragrance and bitter, aromatic taste and yields a blue-coloured volatile oil. This erect annual plant is also called "Pin heads", which alludes to the more pointed appearance of the flowerheads. The species has much smaller flowerheads than Roman chamomile (Wren, 1985 & Craker, 1986).

German chamomile grows in waste places and along road-sides to a height of 20 cm to 90 cm. The leaves are finely cut into linear segments. The numerous daisy-like heads have yellow centres and white rays which have five teeth at the tips (Dwelly, 1977). The flowerheads have a conical, hollow receptacle, with only one row of ligulated florets which are usually bent backwards when dried, and no membraneous bracts (Wren, 1985 & Craker, 1986).

2.1.2.1. Roman chamomile

Roman chamomile, also called English, Garden, Lawn, Sweet, True or Double chamomile, is *Chamaemelum nobile* (formerly *Anthemis nobilis)*. It is also known as "Whig Plant" or "May-then". It is a strongly fragrant, much branched perennial plant (Wren, 1985 & Craker, 1986). Roman chamomile is a creeping or tailing plant with tuft leaves and flowers about 30 cm high. It is a plant with freely branching hairy stems and finely divided leaves. The flowers are daisy-like with white outer ray florets surrounding the

many yellow tubular florets in the centre (Grieve, 1982). The fully open flowers are used medicinally. A blue essential oil is obtained by steam distillation of these flowers. The species is source of the drug, chamomile flower (BP, 1988) *Chamomillae romanae flos* (EP, volume 2).

2.1.2.3. Scentless or wild chamomile

Scentless or wild chamomile (*M. martima)* grows in waste places and is similar to *M. chamomilla,* but the flowers are larger, rays are longer, and there is no odour. It grows in waste places near sea ports and is quite localized. It is usually from 15 cm to 45 cm tall, but the branches are usually in a reclining and horizontally spreading positions. The leaves on this plant are divided into segments which are then divided again into fleshy linear segments (Dwelley, 1977). Scentless chamomile is a noxious weed in Alberta, rather common in the Edmonton region (Moss, 1959).

2.1.2.4. Pineapple weed

Pineapple weed (*M.matricaroides*) is a branching, leafy plant growing in farm yards, along road-sides, and in waste places to a height of from 15 cm to 45 cm. The leaves are a deep yellow-green and are deeply cut and recut. When the plant is bruised, it emits an odour similar to a pineapple. The yellow-green flowers are in conical or pointed heads with green bracts surrounding the base of the head. There

are not any rays as there are on most other members of this family (Dwelley, 1977).

2.1.2.5.Stinking mayweed

Stinking mayweed (*Anthemis cotula)* is a daisy-like plant which grows abundantly in waste places and in barn yards to a height of from 20 cm to 60 cm. The foliage is thrice-cut and has a very strong unpleasant odour. The leaves also cause blisters on hands of farm workers who harvest this with the hay crop. It is also known as Dog-Fennel (Dwelley, 1977).

Figure 1. *Matricaria martima* (photograph by Dr. R.A. Locock)

2.1.3. Geographical source

Both common chamomile plants are native to and cultivated in Southern and Eastern Europe. Chamomile is extensively cultivated in Europe where it is widely used in folk medicine. Chamomile is so highly regarded and so extensively used that it might be labelled the "ginseng" of Europe. Recently, chamomile has become one of the most popular herbal teas in the United States (Tyler,1988).

German chamomile is native to Europe and northern and western Asia. It is extensively cultivated in Hungary, Bulgaria, the former Yugoslavia, Germany, Greece and Egypt (Craker, 1986). German chamomile is also one of the most important among the medicinal plants cultivated in Argentina, where it grows also as a wild plant. It is cultivated with a yield of 600 to 1200 kg of dried flowers per hectare. More than 1200 tonne are exported every year (Padula, 1976).

Roman chamomile is native to southern and western Europe. It has been cultivated in England, Belgium, the U.S., Argentina and other countries (Craker, 1986).

2.2. History, properties and use

Roman and German chamomile have been used in traditional medicine for centuries. They have been known for their medicinal properties since Roman time. A multitude of activities and medicinal uses have been proposed for these

plants and many of these are scientifically justified.

The plants have been used as antispasmodics and sedatives in folk treatment of digestive and rheumatic disorders. Teas have been used to treat parasitic worm infections and as a hair tint and conditioner. The volatile oil has been used to flavour cigarette tobacco (Boulos, 1983).

The flower heads yield bitter and aromatic extracts which have tonic, stomachic, febrifuge, emmenagogue, vermifuge, vulnerary and stimulant properties. Infusions of the plants were used against migraine, jaundice, and digestive troubles. The oil of chamomile was used as a rub for rheumatism (Boulos, 1983).

In addition, the plants were used for the treatment of asthma, convulsions, cough, diarrhea, fevers, gout, toothache, etc. The majority of these uses are only of historic interest. However, some uses have survived to the present, and are scientifically valid (Hamon, 1989).

Matricaria has been the most extensively investigated of the two types of chamomile. It is used everywhere in Europe, almost as a panacea. The Germans refer to it as "alles zytraut" meaning "capable of anything" (Tyler, 1988), a European counterpart to ginseng. The oil has been reported to have bactericidal and fungicidal activity, particularly against bacteria and *Candida albicans* (Aggag, 1972).

An infusion of matricaria flowers has been shown to have a marked hypnotic effect (Leung, 1980). Matricaria tea bags have been used for insomnia, gout, sciatica, indigestion and diarrhea. Matricaria is also said to have a particular place in the treatment of children's ailments, such as colic, teething pains and infantile convulsions (Berry, 1995).

In herbal medical practice and for home use, infusions (made by steeping fresh or dried flowers in water) are taken orally as a digestive aid, for colic, fevers and flatulence, and in large doses as an emetic. Externally, infusions are used as a fomentation or wash for wounds and sores. The flowers are used as poultice (Craker, 1986).

Decoctions, prepared by boiling flowers in water, are used as antispasmodic agents. A rubbing oil made from fresh or dried flowers steeped in olive oil for 24 hours has been used for painful joints and swelling (Craker, 1986).

Extracts of chamomile are used in pharmaceutical preparations, particularly in antiseptic ointments, creams and gels to treat cracked nipples, sore gums, nappy rash and inflammation, and for wound healing. They are also used in cosmetics, bath preparations, hair lighteners, shampoos, sunburn creams and mouthwashes (Leung, 1980).

The extracts can be used to help healing after operations on the large intestine and urogenital systems (Craker, 1986). They also have some sedative effects.

Support for the thesis that the sedative action of orally administered infusions, which induce sleep during cardiac catheterisation in humans is due to the presence of the amino-acid tryptophan, has yet to be documented (Berry, 1995).

The volatile oils are used in carminative, antispasmodic and tonic preparations. They are also used as fragrance components or active ingredients in soaps, detergents, creams, lotions and perfumes.

Both oils and extracts are used as flavours in most major food categories, including alcoholic beverages (bitters, vermouth, benedictine, liqueur), non-alcoholic beverages, frozen dairy desserts, sweet, baked goods, jellies and puddings (Berry, 1995).

Tinctures contain similar chemical constituents to the oils. They are extracted into alcohol and should not be used near the eye (Craker, 1986).

According to the British Herbal Pharmacopoeia, the suggested current use of Roman chamomile are as a carminative, an antiemetic, a spasmolytic, and a sedative, while those for German chamomile are as a carminative, spasmolytic, mild sedative, antiinflammatory, antiseptic and anticatarrhal agent (Hyde,1983).

2.3. Administration and dosage

2.3.1. Preparation

The main active constituents of the chamomiles appear to be concentrated in the volatile oil. This in turn is most abundant in the flower heads and these, or the entire aerial portions of the plants, are used for medicinal purposes. Chamomile is normally administered for internal use as the dried flower heads, infusion (tea) or liquid extract. To obtain maximum benefits, the infusion must be prepared in a closed vessel by steeping chamomile for ten minutes in hot water. This is to preserve and extract as much of the volatile oil as possible, as well as certain water soluble components.

Various types of products are available for the external use of chamomile. These usually contain either the plant extract or the volatile oil and include such things as ointments, shampoos, lotions, inhalations, vapour baths and tooth pastes. The cooled infusion may also be applied locally (Humin, 1983).

2.3.2. Dosage

As mentioned above, preparations of warm and cold infusions of both chamomile and matricaria serve as medicinal agents and as health-related beverages. This represents the largest use of chamomile flowers on the market (Craker, 1986). Teas, made by steeping loose fresh or

dried flowers in water (15 g/240 ml) or tea bags, are used both orally and externally. Unless otherwise prescribed, chamomile flowers (1.5 to 3 g), infusion, liquid extract and tincture are taken orally, three times a day. Matricaria flowers (2 to 8 g) may be used similarly. Liquid extracts (chamomile 1.5 to 3 ml; matricaria 0.5 to 4 ml) are also available, as are tinctures (3 to 5 ml). Tinctures have occasionally been prepared in the home, by extraction with alcohol of different strengths, at a ratio of 20% weight of flowers per volume of alcohol. The constituents of tinctures are similar to those of the alcohol-extracted essential oil (Craker, 1986).

Many of the chamomile products of the German market are made from fluid extracts standardized to a minimum value of chamazulene and α-bisabolol, e.g., Kamillosan®. Semi-solid preparations typically contain 5 to 15% of the drug or equivalent (Berry, 1995).

2.4. Chemical composition

German and Roman chamomile differ in their essential oil and chemical composition, although several flavonoids and other compounds are common to both.

The essential oils and the extracts of chamomile are very complex mixtures made up of many components which belong to various classes of substances.

2.4.1. Essential oils

The quality of a chamomile oil has been generally evaluated on the basis of its blue colour due to the presence of chamazulene, one of its physiologically active components.

The plants contain 0.6 to 1.75% essential oils of the flower heads (Duke, 1985). Chamazulene, an artifact formed during heating, while preparing teas and extracts, and during steam distillation comprises about 5% of the essential oil. Up to 50% of the essential oil consists of bisabolol, an unsaturated monocyclic sesqiterpene alcohol (Redaelli, 1980).

Chamazulene, as mentioned above, is an artifact. It is produced from matricin during heating, via the equally unstable chamzulene carboxylic acid (Heywood, 1977). See figure 2.

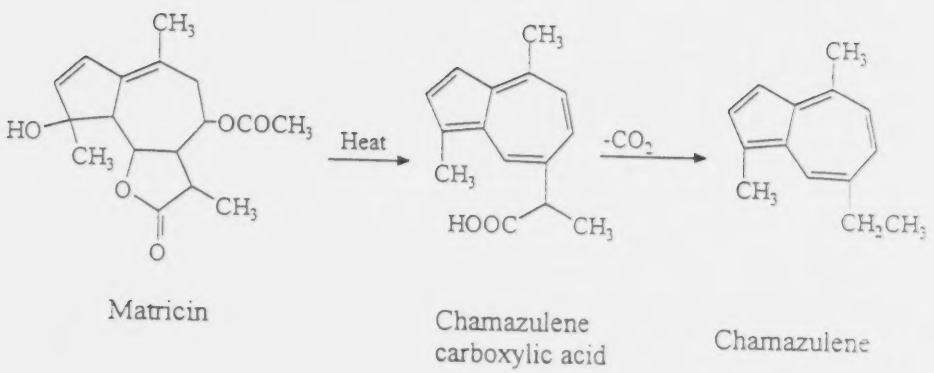
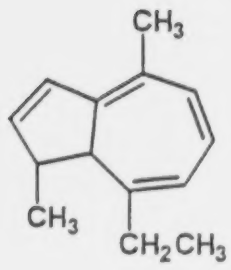


Figure 2. Production of chamazulene

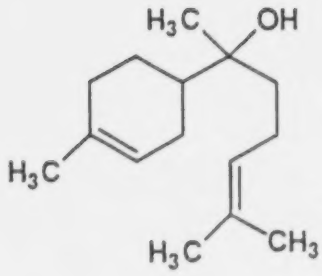
Analysis of chamomile flower oil and extract demonstrated the presence of at least 34 compounds, consisting mainly of esters, terpene hydrocarbons, carbonyl derivatives and waxes. These constituents are listed in table 2 (Cartoni, 1990).

Cartoni and coworkers (1990), using a high efficiency microcapillary GC column for the analysis of essential oils, showed that there are qualitative as well as quantitative differences between a chamomile extract (obtained by extraction with dichloromethane) and the essential oil (obtained by steam distillation): the first contains a large number and amount of unknown higher boiling compounds, while chamazulene and farnesene were not found, and α-bisabolol is present in much lower concentration; the second contains essential oils including α-bisabolol (in a very high concenteration), chamazulene and farnesene.

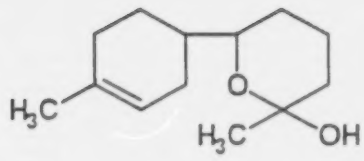
Location, accumulation and composition of the essential oils in different plant parts of chamomile were investigated. Under these investigations the root of the plant was shown to contain the sesquiterpenes chamomillol, caryophyllene, caryophyllenepoxide and the polyeyens chamomilla ester I and II (Reichling, 1984). The structures of some constituents of the essential oil of chamomile are shown in figure 3.



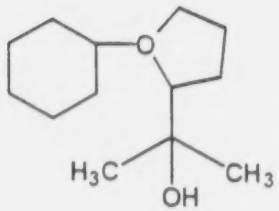
Chamazulene



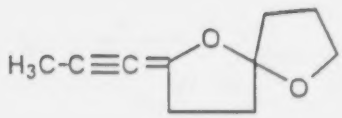
Bisabolol



Bisabolol oxide A

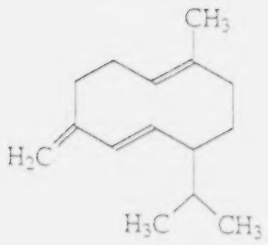


Bisabolol oxide B



En-In-Dicycloether
(Cis-spiroether)

Figure 3. Some constituents of the essential oil of chamomile

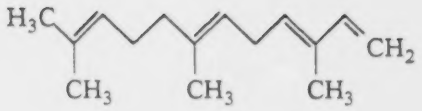


Germacrin

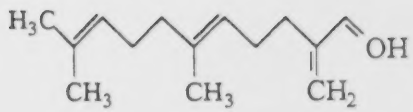
Terpinene

Limonene

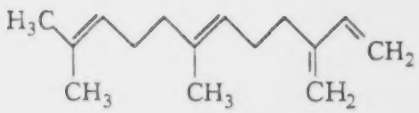
Figure 3. Continued



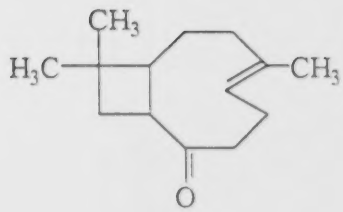
Farnesene



Farnesol



Farnesen



Caryophyllene

Figure 3. Continued

Table 2: Composition of chamomile oil and $CHCl_2$ extract (Cartoni, 1990)

No.	COMPOUND	M.Wt.	Conc.% (OIL)	Conc.% (EXTRACT)
1	α-thujene	136	0.08	0.16
2	α-pinene	136	0.01	----
3	α-phellandrene	136	0.14	----
4	limonene	136	0.54	0.43
5	eucalyptol	136	0.15	0.21
6	gamma-terpinene	136	0.22	----
7	terpinolene	136	0.02	----
8	linalool	154	0.01	----
9	borneol	154	0.03	----
10	α-terpineol	156	0.09	----
11	bornyl acetate	182	0.05	----
12	citronellyl acetate	198	0.02	0.06
13	geranyl acetate	196	0.13	----
14	ß-caryophyllen	204	0.28	----
15	humulene	204	0.03	0.03
16	ß-farnesene	204	18.67	----
17	germacrene D	204	2.72	----
18	α-farnesene	204	5.34	----
19	cis-nerolidol	222	0.13	0.10
20	bisabololoxide	238	4.38	----
21	α-bisabolol	222	37.99	0.14
22	camazulene	194	2.51	----
23	farnesol	222	2.16	1.37
24	camphene	136	----	2.56
25	sabinene	136	----	0.59
26	ß-cymene	134	----	0.29

Table 2. Continued

NO	COMPOUND	M.WT.	C% (OIL)	C% (EXTRACT)
27	terpinene-4-ol	156	----	0.29
28	neral	152	----	0.02
29	geranial	152	----	0.02
30	thymol	150	----	0.02
31	octadecane	254	----	0.06
32	eicosane	282	----	0.05
33	docosane	310	----	0.04
34	tetracosane	338	----	0.09

2.4.2. Phenolic constituents

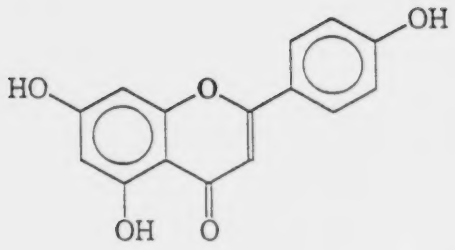
Phenolic compounds are widespread plant secondary metabolites, the flavonoids being the largest group. However, simple monocyclic phenols, phenyl propanoids and phenolic quinones are also frequently encountered.

2.4.2.1. Flavonoids

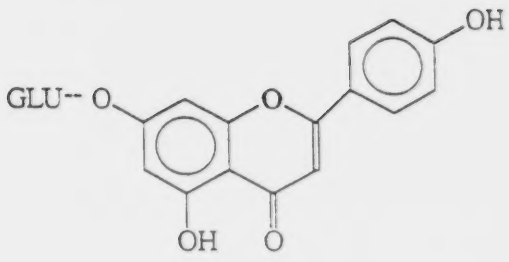
Apigenin (4',5,7-trihydroxyflavon) and its glycoside apigenin-7-O-glucoside, and luteoline (3,4',5,7-tetrahydroxyflavon) and its glycoside luteolin-7-O-glucoside were the earliest flavonoids to be isolated and characterized from the chamomile plants. Besids these known flavonoids, a new one named anthemoside, was isolated. This flavonoid was identified as anthenobilic (sic) ester of apigenin-7-glucoside. Hence on hydrolysis it gives

cosmioside and apigenin-7-glucoside, commonly known as cosmoside.

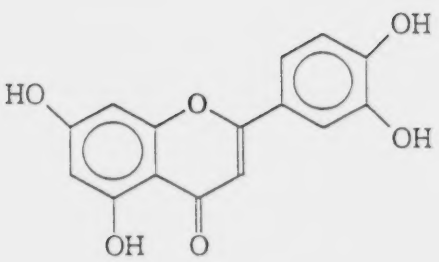
It is important to note that there are marked differences in the chemical composition of the different parts of the chamomile plant flower with respect to these constituents. Accordingly the lingulated white flowers were shown to contain anthemoside and its hydrolysis products, while the yellow tubular flowers were shown to contain luteolin-7-glucoside and free luteolin. The structures of some of the chamomile flavonoids are shown in figure 4.



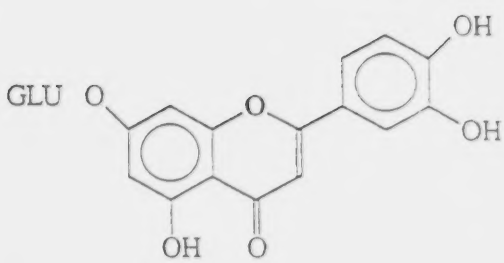
Apigenin



Apigenin-7-O-Glucoside



Luteolin



Luteolin-7-O-Glucoside

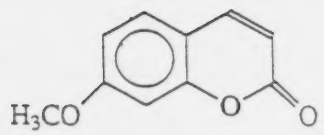
Figure 4. Some flavonoids of chamomile

2.4.2.2. Phenolic acids and coumarins

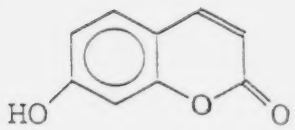
As in the case of the flavonoids, samples of chamomile full flowers were shown to have differences in their phenolic compound composition depending on the part of the flower used. The yellow tubular flowers showed the presence of trans-caffeic acid (3,4-dihydroxycinnamic acid), ferrulic acid (4-hydroxy 3-methoxycinnamic acid), scopoletol, scopolocide and the glucose ester of trans-caffeic acid and ferrulic acid. The white ligulate flowers, on the other hand showed the presence of trans-caffeic acid, scopoloxide, and no more than a trace of free ferrulic acid. This explains why the "double cultivated" variety of chamomile which contains no yellow tubular flowers contains no ferrulic acid.

During the isolation of matricin, the pro-azulene component from chamomile, a considerable amount of mother liquor from the crystallization process was available. The mother liquor showed the presence of three other crystalline compounds. Two of these compounds were isolated and characterized as herniarin (7-methoxycoumarin) and matricarin.

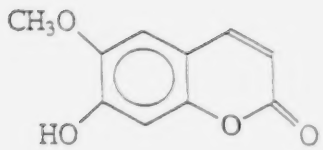
Another coumarin derivative isolated from chamomile was umbelliferone. The oil of chamomile, however, contains mainly (about 85%) aliphatic esters of angelic and tigilic acids (Tyler, 1988). The structures of some of the coumarin derivatives of chamomile are given in figure 5.



Herniarin



Umbelliferone



Scopoletin

Figure 5. Some coumarin derivatives of chamomile

2.5. The pharmacology of chamomile

Unless a chamomile preparation is described in terms of the individual constituents, predictable pharmacological effects may not occur. Countries such as Germany, where medicinal use of chamomile is extensive, have developed standards for the essential oil content of the flower and for the chamazulene and α-bisabolol content of various products. Most studies on the pharmacological properties of both chamomiles have been conducted in animals (Berry, 1995).

2.5.1. Extracts of matricaria

Anti-inflammatory effects of matricaria predominate from external application, whereas smooth muscle relaxing effects predominate from internal adminstration. Smooth muscle relaxant activity resides mainly in the water-soluble components of decoctions. The effects of matricaria in smooth muscle are weaker than the usual therapeutic dose of atropine.

Anti-inflammatory, antipeptic and smooth muscle relaxant effects of matricaria extracts on the stomach and duodenum of humans have been demonstrated through gastric biopsies and cytological studies.

External application of matricaria, shown to change energy dependent processes occurring in the metabolism of dermal cells, could aid cellular regeneration and inhibit inflammation (Craker, 1986). Extracts might be helpful to

patients with mucosal and cutaneous infections. Extracts also inhibit Gram positive micro-organisms, fungi, and streptococcal toxin production.

Dressings containing Kamillosan (in addition to standard treatment of calcium, corticosteroids and histamines) applied to the lower legs of patients with dermatitis, showed anti-inflammatory, deodorant, cooling and slight anaesthetics effects (Berry, 1995).

The well documented pharmacological effects of the chamomile includes antiinflammatory (antiphlogistic), antispasmodic, antibacterial, antifungal and cytostatic action (Hamon, 1989).

2.5.2.Azulenes

The azulenes of both types of chamomile have been documented to be anti-allergenic and anti-inflammatory (Craker, 1986), although their exact mechanism of action is not clear. Azulenes have been shown to prevent allergic seizures in guinea pigs for as long as 60 minutes after administration. It has been suggested that they prevent discharge of histamine from tissues by activating the pituitary-adrenal system to release cortisone. As the cortisone prevents the action of fibrinolysis, which initiates histamine release, an anti-allergenic activity results. The anti-inflammatory effects of azulenes have been demonstrated in several animal models. Oral prochamazulene

(matricin) has proved effective. The activities of chamazulene and guaiazulene in a carrageenin inflammation test, 2-3 hours after application, are about equal to each other but they are less therapeutically active than proazulene.

Azulene compounds are known to stimulate liver regeneration. Subcutaneous treatment of partially hepatectomized rats with azulene compounds, including guaiazulene, will initiate formation of new tissue (Craker, 1986).

Chamazulene has been shown to have pain-relieving, wound healing and antispasmodic properties in animal models (Berry, 1995).

2.5.3. α-Bisabolol

Alpha-Bisabolol, a major component of matricaria, is reported to have anti-inflammatory, antibacterial, antimycotic and ulcer protective properties, with low toxicity. In animal models, healing times for stomach ulcers induced by chemical stress or heat coagulation, are reduced. Ulcer development by indomethacin, stress, and ethanol is also inhibited by α-bisabolol. In vitro studies have demonstrated a dose-dependent antipeptic activity, and bisabolol and its oxides are generally considered to be smooth muscle relaxants (Craker, 1986).

2.5.4. Spiroethers

Both cis and trans-en-yn-dicycloethers occur in the oil of matricaria. The cis form inhibits development of dextran-induced edema and decreases plasmakininogen production in rats. It does not however, inhibit local edema caused by injection of serotonin, histamine or bradykinin, or decrease anaphylactic shock, but does demonstrate a non-linear, dose-dependant smooth muscle relaxant effect. A local inflammatory reaction is produced by injection into the blood stream (Craker, 1986).

2.5.5. Sesquiterpenoids

Nobilin and its epoxy and dehydro derivatives have demonstrated antitumour activity against human tumour cells in vitro; the activity of hydroxyisonobilin at very low level makes it worthy of further examination (Craker, 1986). Nobilin also displays anti-inflammatory action in animals (Berry, 1995).

2.5.6. Flavonoids and coumarins

These are found in matricaria, and must be considered as having active medicinal properties. Flavonoid glycosides and several flavones (apigenin, quercetin, etc.) are smooth muscle relaxants. The coumarins herniarin and umbelliferone are reported to have minor smooth muscle relaxant activity. Many flavonoids have antiviral activity and coumarins have

antibacterial properties (Craker, 1986).

2.5.7. Antimicrobial activity of chamomile

The antimicrobial property of chamomile oil was tested against Gram positive and Gram negative bacteria, as well as a fungus, *Candida albicans*. The Gram positive bacteria used for the test were represented by *Staphylococcus aureus* and *Bacillus subtilis*.The tested Gram negative bacteria were *Escherchia coli* and *Pseudomonas aeruginosa*. The result showed that oil concentrations above 0.05% exerted marked bacteriostatic and bactericidal effects against the tested Gram positive bacteria and the fungus. Concentrations below 0.025% showed, however, showed no antimicrobial activity. On the other hand the Gram negative organisms were relatively less sensitive to the action of the oil (Aggag, 1972). The effect of chamomile oil on the above mentioned organisms is shown in table 3 (Gram postive) and table 4 (Gram negative).

Table 3. Effect of chamomile oil on Gram postive bacteria and *C. albicans* (Aggag, 1972)

% Chamomile oil in broth v/v	Number of survivors/ml of medium (after 24 hr. incubation)		
	S.AUREUS	B.SUBTILIS	C.ALBICANS
0	$1.30X10^{9}$	$8.32X10^{6}$	$1.58X10^{7}$
0.025	$1.42X10^{9}$	$7.94X10^{6}$	$1.57X10^{7}$
0.05	$5.78X10^{8}$	$5.01X10^{6}$	$1.05X10^{7}$
0.10	$3.95X10^{8}$	$2.34X10^{6}$	$4.73X10^{6}$
0.20	$1.47X10^{8}$	$1.58X10^{5}$	$6.31X10^{5}$
0.30	$1.77X10^{7}$	$6.61X10^{3}$	$4.17X10^{4}$
0.40	$2.14X10^{5}$	$4.96X10^{2}$	$3.63X10^{3}$
0.50	$6.49X10^{3}$	4.90X10	$3.38X10^{2}$
0.60	$1.99X10^{2}$	0.00	3.90X10
0.70	0.00	0.00	0.00
Initial inoculum	0.00	0.00	0.00

In comparing the antimicrobial activity of the individual isolated components, chamazulene was reported to possess no significant activity both on Gram positive and Gram negative bacteria or Candida. The constituent with the greatest antibacterial effect was shown to be (-)-α-bisabolol. The antifungal activity against *Candida albicans*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* was attributed to (-)-α-bisabolol and En-In-dicycloether (Szalontia, 1977).

Table 4. Effect of chamomile oil on Gram negative bacteria (Aggag, 1972)

Table 4. Effect of chamomile oil on Gram negative bacteria (Aggag, 1972)

% Chamomile oil in broth v/v	Number of survivors per ml of medium (after 24 hr.incubation)	
	E. COLI	**P. AERUGINOSA**
0	$3.36X10^{9}$	$4.36X10^{8}$
1	$2.60X10^{9}$	$4.27X10^{8}$
2	$6.49X10^{8}$	$2.51X10^{8}$
3	$3.16X10^{8}$	$1.26X10^{8}$
4	$2.60X10^{8}$	$1.05X10^{8}$
5	$2.54X10^{8}$	$2.51X10^{7}$
6	$1.53X10^{8}$	$5.01X10^{6}$
7	$7.94X10^{7}$	$2.27X10^{6}$
8	$4.13X10^{7}$	$2.75X10^{6}$
Initial Inoculum	$1.42X10^{5}$	$1.77X10^{5}$

2.5.8. Anti-inflammatory and spasmolytic properties of chamomile

The true chamomile is one of our oldest pharmaceutical plants. Aqueous and alcoholic extracts have been used since antiquity internally and externally, for their anti-inflammatory and wound healing activity (Glowania, 1987; Herz, 1977).

For a long time the only known active principle was the blue azulene compound chamazulene, which is produced from matricin during steam distillation. More recently, as a result of systematic research, other active substances have been found, which posses even greater activity than

azulenes. The first compound was (-)-α-bisabolol an unsaturated monocyclic sesquiterpine alcohol; the dextrorotatory form does not exist in chamomile. α-Bisabolol can occur in the isopropylidine or in the isopropenyl form. It was a more efficient antiphlogistic agent than guaiazulene when tested against the carrageenin edema of rat paw. Laevorotatory bisabolol is a more powerful antiphlogistic and spasmolytic agent than the dextrorotatory form or the racemate (Issac, 1974, 1977).

Two other compounds, bisabolol oxide and the spiro ether, have also been found in the oil of chamomile. The most abundant cis-spiroether has a better antiphlogistic activity than chamazulene. The spasmolytic activity of cis-spiro ether was found to be superior to that of papaverine (Breinlich, 1968).

The drug also contains a high proportion of spasmolytically active flavone glycosides such as apigenin (Della-Loggia, 1988). Thus azulene no longer ranks as the sole substance responsible for the pharmacological properties of chamomile.

Many reports show that chamomile preparations are widely used in popular medicine to reduce cutaneous and mucosal inflammations. Clinical uses of chamomile preparations in dermatological disease are also described (Isaac, 1980). In both cases topical administration is preferred. The antiphlogistic action of chamomile after

systemic administration has been widely demonstrated in a variety of experimental inflammatory reactions in animals, both for crude extracts and for some pure components. Rossie (1988) studied the anti-inflammatory effect of chamomile using the plantar edema induced by carrageenin. Their finding showed that the essential oils of chamomile exert a considerable effect, particularyly 3 hours after injection.

In an attempt to elucidate the anti-inflammatory effects, Hall et al. (1980), have conducted extensive research in rat and mouse liver cells. The results showed that the anti-inflammatory action of sesquiterpene lactones resembeled that of currently used drugs in that they were potent inhibitors of neutrophil migration, lysosomal rupture, enzymatic activity, and prostaglandin synthesis, which was linked to elevated cyclic adenosine monophosphate levels.

Tubaro et al. (1984), on the other hand, checked the antiphlogistic activity of a chamomile extract after topical application. In this study the croton oil ear test was carried out using hydroalcoholic extract of *Chamomilla recutita*. Benzydamine served as reference drug. The hydroalcholic extract, according to the study, induced a reduction of the croton oil edema similar to that obtained with the non-steroidal anti-inflammatory agent used as a reference, and the ED_{50} value of the hydroalcoholic extract is similar to that of benzydamine. From the presented data

it can be deduced that *C. recutita* exerts its anti-inflammatory activity after topical application. Since the croton oil induces a vascular (dermal) dermatitis (Swingle, 1981), it can be suggested that the chamomile preparation acts at this level as well.

More detailed understanding of the physiology of the mechanism of muscle contraction has allowed differentiation between mode of action of the varied constituents of a number of plant extracts. It was previously mentioned that chamomile shows spasmolytic activity due to, among other compounds, to α-bisabolol and apigenin. Using a technique developed for the specific evaluation of calcium antagonism based on the depolarization-induced contraction of rabbit aortic rings, it has been possible to differentiate between the types of activity of apigenin, a flavonoid which shows no activity in the test, and α-bisabolol, which was a potent inhibitor of KCl-induced contraction. Thus α-bisabolol and not apigenin is a calcium antagonist (Vuorela, 1985).

2.6. Commercial preparations

In addition to medicinal applications, extracts of the chamomiles are used as scent enhancers in several cosmetic products, nonirritating dyes, and occasionally, as flavouring agents in alcoholic beverages. The essential oil of Roman chamomile is added as a trace ingredient to perfumes to give a fresh, warm note and depth. The essential

oil of German chamomile is also added to soaps, lotions, and creams (Furia, 1975; Opdyke, 1974).

The low toxicity and yellow-colouring agents (apigenin, apigenin glucoside, and other flavonoids) in both Roman and German chamomile flowers have promoted their use in vegetable hair dyes. The resultant hair colour following use of chamomile-based dyes depends upon the original hair colour and the length of application. Repeated applications of chamomile to any hair colour are reported to produce a brilliant yellow. For a less glaring yellow colour, chamomile is mixed with henna, *Lawsonia inermis* L. (Craker, 1986).

2.7 Toxicity of chamomile

The toxicity of chamomiles, their extracts and (-)-α- bisabolol is apparently very low (Habergang, 1979). No teratogenic effects have been observed. It has been claimed that chamomile tea might cause anaphylaxis, bronchitis and dyspnoea and that enema preparations might cause asthma and urticaria. Similarly, contact with the plant or use of chamomile ointments has been claimed to produce dermatitis (Hausen, 1992; Van-Ketel, 1987).

The principal adverse effect appears to be hypersensitivity resulting from exposure to the pollen (Benner, 1973; Casterline, 1980; Subiza, 1990).

The Asteraceae (Compositae) are one of the largest

families of flowering plants, comprising about 25,000 species. The real offenders of this family are the wind pollinated plants that include the subtribe Ambrosiine (Ambrosia, Dicoria, Hymenoclea, Iva, Xanthium, Parthenice, some Baccharis and Artemisia). In North America, the species of Ambrosia are by far the most important cause of pollinosis, with *A. artemisiifolia* and *A. trifda* alone accounting for more hay fever than all other plants together (Lewis, 1983). Most Compositae pollen is potentially allergenic; pollens that are most closely allied to ragweed and other Ambrosiinae pollens are particularly allergenic (Leiferman, 1976).

The plant chamomile also belongs to the Compositae family, but unlike the other previously mentioned members of the family, its pollination is entomophilous. Although the infusion made from its flowers is a common beverage all over the world because of, in part, the numerous therapeutic properties attributed to it, very few cases of anaphylaxis after ingestion have been described (Lewis, 1983). In one of these reported anaphylactic cases, a cross-reactivity between Matricaria and Artemisia pollens, was used to explain a mechanism of indirect sensitization. The cross-reactivity is not surprising because both plants have a close taxonomic relationship. This two plants belong to the same Compositae family and to the same tribe, Anthemideae. The pollination of Matricaria plants demonstrate

entomophilous, whereas the Artemsia plants demonstrate anemophilous pollination, and a significant concentration of this pollen is found in the atmosphere. It was strongly suggested and proven that the allergic activity of Matricaria in the reported case, was actually caused by its cross-reactivity with Artemisia pollen, which appeared to be the sensitizing agent. Likewise, cross-reactivity could also account for the reactivity of an individual to the pollen of *A. trifid* (Subiza, 1989).

The findings presented here might indicate that patients with hay fever, caused by sensitization to Compositae pollens (rag weed or mugwort), should also be investigated to rule out sensitization to *M. chamomile* because of the potential hazard of its infusion to these individuals.

The majority of reports of allergic contact dermatitis refer to *Anthemis cotula* (the stinking dog-fennel) and related species. This species is known for its high content of the allergenic sesquiterpene lactone, anthecotulide. Only traces of this lactone are present in matricaria or chamomile, if present at all. Nontheless some sesquiterpene lactones are allergenic, or potentially so, a prerequisite being the possession of an exocyclic α-methylenic group, and rare instances of allergic response and anaphylaxis are recorded for chamomile. The proper identification of source material for chamomile products is essential if the number

of such reactions is to be minimised (Hausen, 1992).

Even though Ambrosia pollen is the most important cause of hay fever in North America and the infusion of chamomile is a common beverage, it is remarkable that very few cases of allergy to the latter have been described (Subiza, 1989).

CHAPTER III

EXPERIMENTAL

3.1. Instruments

1. Automatic fraction collector
   (Buchler Fractomette 220, Fort Lee, New Jersey)

2. Sartorius Electronic Analytical Balance
   (Sartorius Instruments Ltd., Surrey, Great Britain)

3. Buchler Flash Evaporator
   (Buchler Instruments, Fort Lee, New Jersey)

4. Disc Mill S. 500
   (Glen Mills, Clifton, New Jersey)

5. Glass column
   (Kontes Glass Co., Vineland, New Jersey)

6. Heavy duty blender
   (Waring Products Division, New Hartford, Connecticut)

7. Hot-stage melting point apparatus
   (Mettler Instruments Corporation, Princeton, New Jersey)

8. Infrared spectrophotometer
   (Nicolet FT, Nicolet Instrument Corporation,
   Madison, Wisconsin)

9. Mass spectrophotometer
   (AEI-MS-50 Mass Spectrophotometer, Associated
   Electrical Industries, Manchester, England

10. Nuclear magnetic resonance spectrophotometer
   (Bruker AM 300 FT, Spectrospin, Toronto, Canada)

11. Reagent sprayer
   (CAMAG, Schott, Berlin, West Germany)

12. Thin-layer chromatogram chamber
   (Eastman Kodak Co., Rochester, New York)

13. Thin-layer chromatogram glass developing tank
   (CAMAG, Schoott, Berlin, West Germany)

14. Triple beam balance
   (Ohaus Scale Corp. Union, Florham Park, New Jersey)

15. Ultra-violet lamp
   (Mineralight UV lamp model UVGL-58, Ultra-Violet
   Products Inc., San Gabriel, California)

16. Ultra-violet/Visible spectrophotometer
    (Philips PU8740, Pye Unicam Ltd., Cambridge, Great Britain)

3.2. Materials

1. Aluminium chloride
   (BDH, Dorset, England)

2. Apigenin
   (Sigma Chemical company, St. Louis, Missouri, U.S.A)

3. Diatomaceous earth
   (Celite® 545, Fisher Scientific, Ottawa, Ontario)

4. Filter paper
   (Whatman, Clifton, New Jersey)

5. Air-dried and powdered flowers of scentless chamomile (*Marticaria martima*), collected in Jul, 1993 and 1994 from the river valley in Edmonton, Alberta, collected by Dr. R.A. Locock and M. Bekele

6. Glass wool
   (Owens-Corning Fiberglas Co., Corning, N.Y)

7. Pre-coated analytical silica gel TLC plate with fluorescent indicator, 0.10 mm thickness
   (Eastman Kodak Co., Rochester, New York)

8. Silicic acid 100 mesh
   (Mallinckrodt Chemical Works, Pointe Claire, Quebec)

9. Sodium hydroxide
   (BDH Dorset, England)

10. Washed sea sand
    (Fisher Scientific, Ottawa, Ontario)

3.3. SOLVENTS

1. Ethanol (95%)

   (Commercial Alcohols Inc., Montreal)

2. Methanol

   (Mallinckrodt, Chesterfield, Missouri)

3. Chloroform

   (Mallinckrodt, Chesterfield, Missouri)

4. Dimethyl Sulfoxide-d6

   (Sigma chemical company, St. Louis, Missouri, U.S.A)

5. Ethyl acetate

   (Anachemia, Mississauga)

6. Hexane

   (BDH, Dorset, England)

7. Hydrochloric acid, 36.5-38%

   (BDH, Dorset, England)

8. Petroleum ether (60-70^{0}) C

   (BDH, Dorset, England)

3.5. Extraction

To one Kg. of dried and powdered scentless chamomile, a sufficient amount of solvent (5 L) composed of methanol and water (9:1) was added and a liquid slurry was prepared. The mixture was left for 24 hrs. Filtration, to separate the extract from the plant material was carried out first by using a glass wool plug in the neck of a filter funnel, followed by suction through filter paper using a Buchner funnel. The mark was again extracted in the same way using 3L of methanol and water in the ratio of 1:1. The two extracts were then combined and evaporated under reduced pressure to about 1/3 the original volume (i.e. until most of the methanol was removed). The resultant aqueous extract was then cleared of low polarity contaminants such as fats, terpenes, chlorophylls, xanthophylls, etc. by repeated extraction (100 ml x 25 times) in a separatory funnel with 2.5 L of hexane. The hexane extract was then evaporated to dryness under vacuum at 40^0 C and weighed. The extraction scheme for the plant material s shown in figure 6.

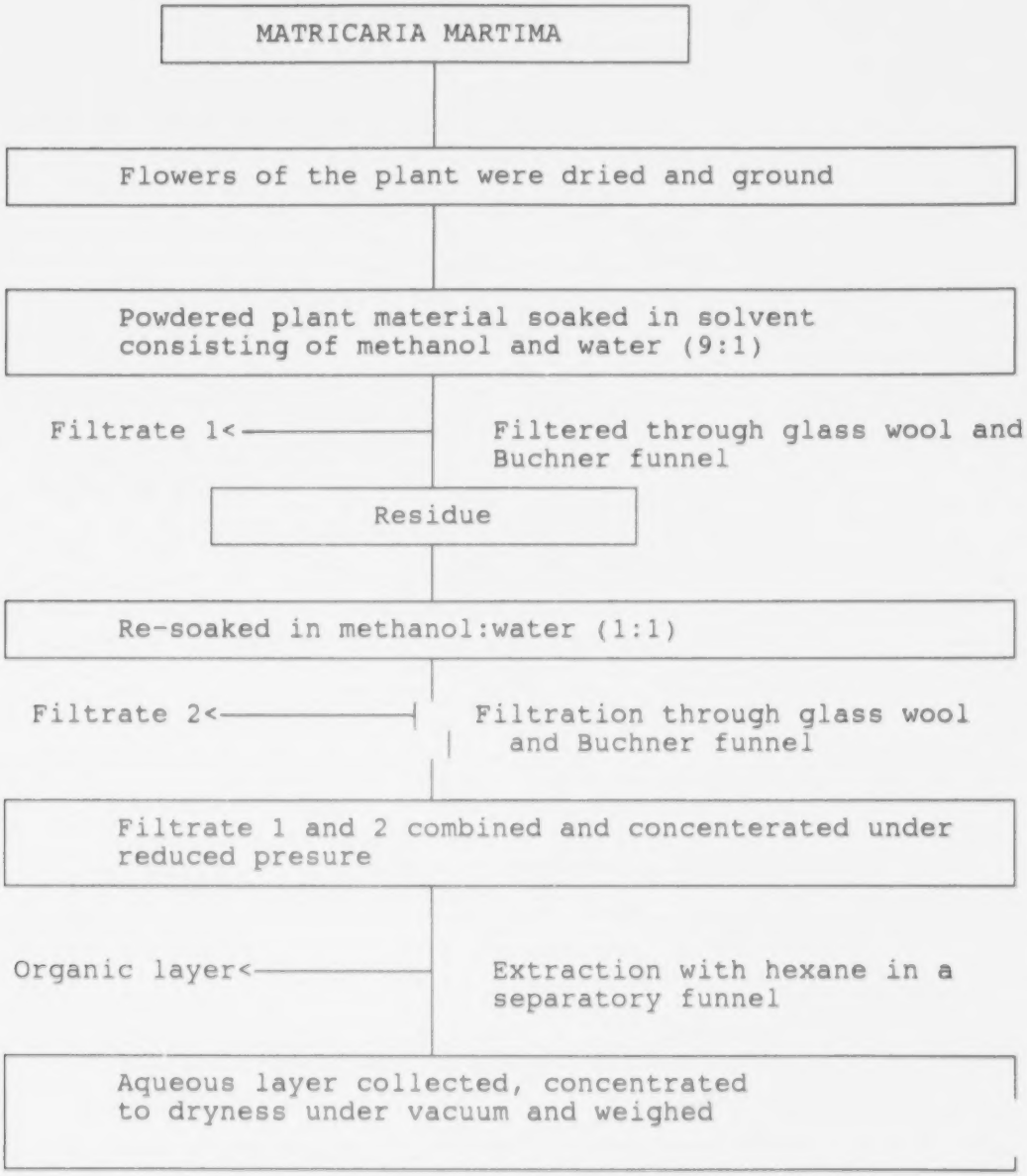

MATRICARIA MARTIMA
Flowers of the plant were dried and ground
Powdered plant material soaked in solvent consisting of methanol and water (9:1)
Filtrate 1
Filtered through glass wool and Buchner funnel
Residue
Re-soaked in methanol:water (1:1)
Filtrate 2
Filtration through glass wool and Buchner funnel
Filtrate 1 and 2 combined and concenterated under reduced presure
Organic layer
Extraction with hexane in a separatory funnel
Aqueous layer collected, concentrated to dryness under vacuum and weighed

Figure 6. Extraction scheme for scentless chamomile

3.6. Chromatographic analysis

3.6.1. Column chromatography

The plant material was taken up in methanol and subjected to column chromatography under the following specifications: internal diameter of column, 1.98 cm; weight of adsorbent material, 60 gm; length of adsorbent matrix, 38 cm; composition of the adsorbent, silicic acid and Celite (8:3).

The packing material was prepared by mixing silicic acid and Celite in the ratio of 8 to 3 with 100 ml of chloroform. This slurry was gravity packed into a chromatographic column which was previously plugged with glass wool. Thirty ml of chloroform was added to the column before packing. After packing, excess solvent was allowed to drain through the column outlet, leaving enough solvent to cover the column surface. The surface of the column was covered with a small amount of washed sea sand.

About 5 gm of the crude extract in methanol was applied to the column. After the sample migrated into the packing material, more chloroform was added on top of the adsorbent surface. The flow rate of the solvent was manually adjusted to 1 ml per minute and the column was run by gradient elution using chloroform-methanol in the ratios given in table 5. Fractions of 10 ml were collected. Each fraction was evaporated to dryness under reduced pressure and re-dissolved with a few drops of methanol for thin layer

chromatographic analysis.

Table 5. Solvent composition for the column chromatography of the crude extract of *M. martima* flowers

Chloroform (%)	Methanol (%)	Total volume (ml)
100	0	200
97	3	200
95	5	200
90	10	200
85	15	200
80	20	200
75	25	200
70	30	200
60	40	200
50	50	200
40	60	200
30	70	200
20	80	200
10	90	200
0	100	200

3.6.2. Analytical thin layer chromatography

Pre-coated TLC plates (silica gel, 0.1 mm) were used for the qualitative analysis. The fractions from the silicic acid column were dissolved in a minimum amount of methanol and spotted 3 cm from the bottom of the TLC plate 1 cm apart from each other. A mobile phase containing ethyl acetate:methanol:water in the ratio of 100:16.5:13.5 was used to develop the chromatogram. Detection of each component was performed by using by ultraviolet (UV) light before and after spraying the with 2% aluminium chloride solution in methanol (Harborne, 1975). Fractions which gave the same UV result and Rf values in the TLC analysis were combined for further analysis. The fractions combined and the respective Rf values of their components are given in tables 6 and 7.

Table 6. Composition of fractions from column chromatography of *M.martima* flowers extract

Fraction No.	Component*														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6-21	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
22-37	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-
38-53	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-
54-130	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
130-160	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+

+ = a component is present

- = a component is absent

* TLC characteristics of these components are described in table 7

Table 7. Rf Values and UV/$AlCl_3$ colour reactions of fractions from the column chromatography *M. martima* flowers extract.

Compon.	UV (Before spray)	UV (after spray)	Rf
A	-----	yellow fluorescence	0.97
B	quenching of back-ground fluorescence	quenching of back-ground fluorescence	0.88
C	purple fluorescence	pink fluorescence	0.81
D	yellow fluorescence	yellow fluorescence	0.77
E	blue fluorescence	blue fluorescence	0.73
F	quenching of back-ground fluorescence	quenching of back-ground fluorescence	0.65
G	-----	yellow fluorescence	0.61
H	-----	yellow fluorescence	0.59
I	-----	yellow fluorescence	0.54
J	-----	yellow fluorescence	0.51
K	blue fluorescence	blue fluorescence	0.41
L	-----	yellow fluorescence	0.27
M	-----	yellow fluorescence	0.18
N	-----	yellow fluorescence	0.11
O	-----	yellow fluorescence	0.06

Fraction 6-21 was subjected to column chromatography with a solvent system whose composition is given in table 8 under the following chromatographic condition: internal diameter of column, 1.07 cm; weight of adsorbent material, 10.5 gm; composition of adsorbent silicic acid and Celite (8:3); length of the adsorbent, 23 cm.

Table 8. Solvent composition for the column chromatography of fraction 6-21

Chloroform (%)	Methanol (%)	Total volume (ml)
100	0	100
99	1	100
98	2	100
97	3	100
96	4	100
95	5	100

TLC analysis of the fractions monitored by UV light and $AlCl_3$ spray reagent were used to combine fractions with similar spot characteristics as shown in table 9.

Table 9. Composition of fractions from the column chromatography of fraction 6-21

Fraction No.	Compound				
	1	2	3	4	5
I (1-10)	-	-	-	-	-
II (11-31)	+	+	-	-	-
III (32-49)	-	+	+	+	+
IV (50-60)	-	-	+	+	+

+ = a compound is present
- = a compound is absent

3.6.3. Preparative thin layer chromatography

Preparative TLC plates were prepared with silica. A slurry of finely divided silica in water (1:2) was spread on 20 x 20 cm glass plates to a thickness of 0.75 mm using a spreader of the specified thickness. The plates were air-dried at room temperature and activated in an oven at 110^0 C for one hour.

Fraction II (11-31) was applied to the preparative TLC plate as a band 3 cm from the bottom of the plate. The chromatogram was developed using ethyl acetate, methanol and water (100:16.5:13.5) as a solvent. The two separated bands, identified by UV light and $AlCl_3$ detecting reagent, were scraped from the plate and the constituents were extracted into methanol. Filtration was used to separate the

components from the silica gel. Each band was spotted in a pre-coated TLC plate and confirmed to be a single spot. The filtrates were then concentrated to dryness by evaporating under reduced pressure. Each component was crystallized from ethanol. After repeated recrystallization and drying their respective melting points were determined using a hot-stage melting point apparatus.

The same procedure was performed for fraction IV (50-60), and three separate bands were obtained. Fraction III was found to be a mixture of II and IV. Therefore, it was not further processed.

3.7. Spectrophotometric analysis

3.7.1. Ultraviolet spectroscopy (UV)

The samples were prepared by dissolving a small amount of the isolated compounds in methanol. The optimum concentration for UV analysis was determined by preparing serial dilutions of the stock solution. The UV spectrum for each of the isolated compounds was scanned in the range from 200 nm to 500 nm with methanol as a blank. After measuring the spectrum of the samples in methanol (the "MeOH" spectrum), six drops of 1% $AlCl_3$ reagent were added, mixed, and the "$AlCl_3$" spectrum was measured. Finally HCl (3 drops) was added and "$AlCl_3$/HCl" spectrum was measured.

The UV absorption of these compounds was also measured after the addition of 2M NaOH to the methanol solution.

3.7.2. Nuclear magnetic resonance spectroscopy (nmr)

^{1}H NMR (300 MHZ) spectra were recorded for each of the isolated compounds. The samples were prepared by dissolving 2 mg of each in sufficient amount of hexadeuterodimethyl-sulphoxide (d_6-DMSO). The NMR spectra were run by Dr.V. Somayaji.

3.7.3. Mass spectroscopy (MS)

The crystallized samples were directly used to obtain the mass spectra of each of the isolated compounds. Ions were produced by electron bombardment of the compounds in the ion chamber and ion separation was achieved by means of electromagnet. The MS was performed by Mr. L. Harrower.

CHAPTER IV

RESULT AND DISCUSSION

4.1. Extraction and isolation

The Chamomile flower contains numerous organic compounds which vary considerabely in their chemical and structural characteristics. Constituents can be obtained either by extraction or steam distilation. Extraction with a mixture of methanol and water yielded considerable amount of these substances. After washing these substances with petroleum ether and evaporating to dryness the methanol/water extract to dryness at 40°C under reduced pressure, the residue was weighed accurately to give 260.62 gm of the total extract. The percent yield of the petroleum ether washed methanol/water extract of the dry flower was 0.261 % w/w.

The TLC analysis of the fractions collected from the column chromatography showed the presence of fifteen different compounds. From these, five of the compounds which were present in fraction 6-21 were separated into two other fractions by subsequent column chromatography. The resulting fractions [i.e. I (11-31) and IV (50-60) which contain 2 and 3 compounds respectively], were subjected to preparative TLC to isolate the individual components. Fraction I with no component, and fraction III which is a mixture of II and IV, were not subjected to preparative TLC.

4.2. Melting point analysis

Three of the five compounds isolated (compound # 1,2, and 3) melted sharply. Melting points are given in table 10. The melting points of compound # 4 and # 5 were not measured as they exceeded 300°C (the maximum temperature of the hot-stage melting point apparatus).

The melting point of compound # 3 matched that of scopoletin (7-hydroxy-6-methoxycoumarin) (204-205°C) (Murray, 1982).

The melting point of compound # 1 matched that of tomentin, 185° C (Murray, 1982).

Melting point matches for compounds # 2, # 4 and # 5 with literature values were not found.

Table 10. Melting point data for the five isolated components of *M. martima* flowers

Compound	Melting Point (°C)
#1	185-187.5
#2	245-247
#3	205
#4	> 300
#5	> 300

4.3 Identfication of compound # 4

Based on the characteristic features observed in TLC, ultraviolet absorption spectroscopy, nuclear magnetic resonance spectroscopy and mass spectroscopic studies, compound # 4 was identfied as apigenin.

4.3.1 Ultraviolet spectroscopy analysis of compound #4

The "MeOH" spectrum of compound # 4 gave two distinct bands. Band II at 268.7 nm (ϵ = 18,802, log ϵ = 4.27) originates from the A-ring benzoyl system and Band I at 336 nm (ϵ = 20,933, log ϵ = 4.32) from the B ring cinnamoyl system (Harborne, 1975). See table 11 for the absorption bands and appendix I for the spectrum.

Table 11. UV absoption peaks (nm) for compound # 4

Band	"MeOH"	"$AlCl_3$"	"$AlCl_3$/HCl"
I	336	384	379
II	268.7	347	343

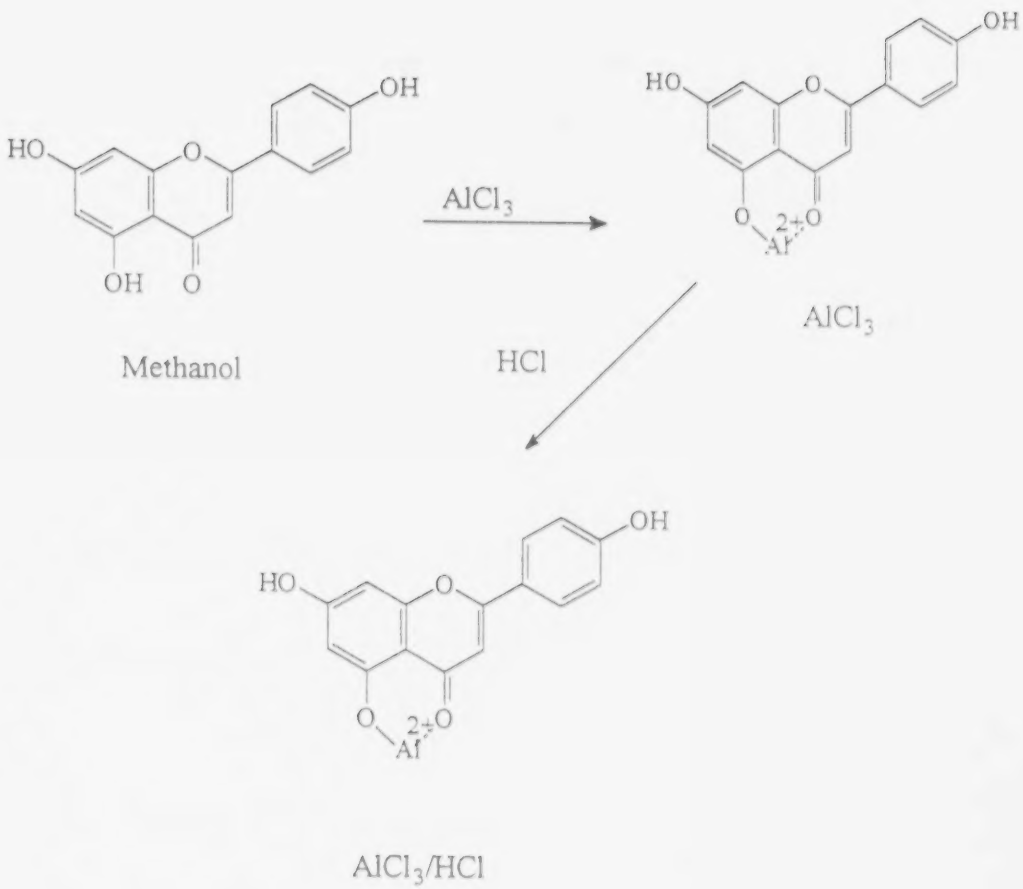


Figure 7. Suggested structures for Apigenin in different media

The pattern of hydroxyl groups substitution and the relative position of the keto group on compound # 4 was studied by its "$AlCl_3$" and "$AlCl_3$/HCl" spectra. "$AlCl_3$" can form acid-stable complexes between hydroxyl groups and neighbouring ketones. The complex "$AlCl_3$" form between *ortho*-dihydroxy groups is acid labile and can easily be hydrolyzed by HCl. "$AlCl_3$" and "$AlCl_3$/HCl" were used to detect both groupings. The "$AlCl_3$" spectrum represent the sum total effect of all complexes on the spectrum. The "$AlCl_3$/HCl" spectrum on the other hand represents the effect only of the hydroxy-keto complex (Markham, 1982). See figure 7.

The "$AlCl_3$" spectrum of compound # 4 showed a bathochromic shift from 336 nm to 384 nm for Band I. This increase of 48 nm is an indication of the absence of a dihydroxy substituted B-ring. For such substitution the expected increase in absorption is 65-95 nm (Markham, 1982).

The "$AlCl_3$/HCl" spectrum also showed a bathochromic shift of 43 nm for Band I. This is an indication of the presence of a 5-OH group in the A-ring. The "$AlCl_3$/HCl" spectrum of 5-hydroxy substituted flavones shows an increase of 35-55 nm for Band I (Markham, 1982).

4.3.2. Mass spectroscopy analysis of compound # 4

The high resolution mass spectroscopy of compound # 4 gave seven relatively prominent peaks (Table 12). The base peak was observed at m/z 60 (formula $C_2H_4O_2$). The m/z 60 peak is also present in the spectra of the authentic sample of apigenin. By considering m/z 270 as the base peak, the fragmentation pattern of compound # 4 was studied (Fig.8).

The first fragment ion to be considered is the $[M-H]^+$ ion which is obtained by loss of a hydrogen atom from the hydroxyl group of ring B. The intact molecular ion also gave m/z 242 (i.e.[M-28]) by losing carbon monoxide.

The most useful fragmentations for characterization involve cleavage of the intact A-ring and B-ring fragments. They undergo cleavage by two different pathways. The first pathway is retro-Diels Alder (RDA) which yeilds contains A-ring and C-ring fragments (i.e. m/z 152 and m/z 153). The m/z 153 ion is an RDA product which in addition involve hydrogen transfer. The second pathway produces a B-ring and a C-ring derived fragment of m/z 121.

Loss of a carbon monoxide group from m/z 152 gave another important fragment ion of m/z 124 (Harborne, 1975; Markam, 1982). See appendix II for the mass spectra.

Table 12. Relative abundance of some important fragment ions of compound #4

m/z	% relative abundance	Formula
270	55.44	$C_{15}H_{10}O_5$
269	6.07	$C_{15}H_9O_5$
242	7.19	$C_{14}H_{10}O_4$
153	15.47	$C_7H_5O_4$
152	10.99	$C_7H_4O_4$
124	6.33	$C_6H_4O_3$
121	9.55	$C_7H_5O_2$

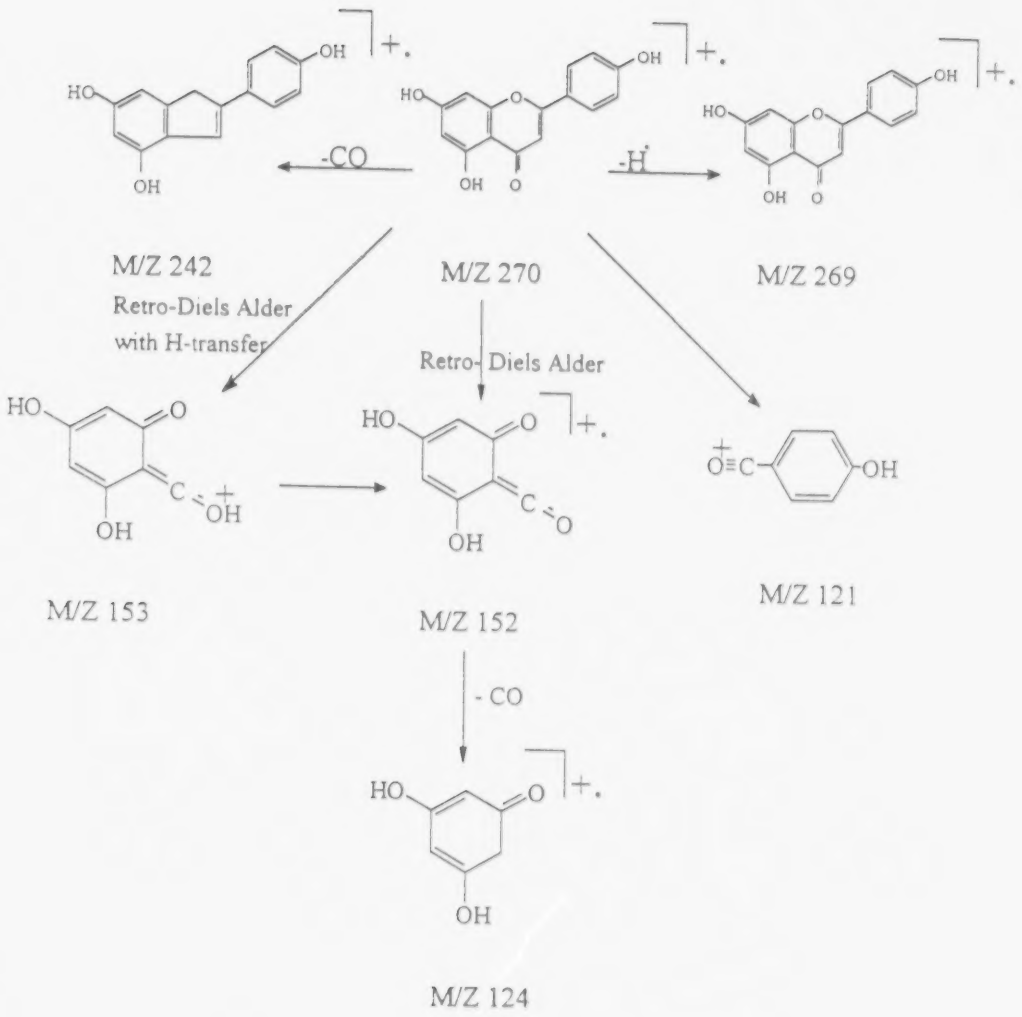


Fig.8 Mass fragmentation pattern of compound # 4.

4.3.3. Nuclear magnetic resonance spectroscopy analysis of compound # 4.

The ^1H NMR signals of compound # 4 were assigned as shown in Table 13.

TABLE 13. ^1H NMR CHEMICAL SHIFTS FOR COMPOUND # 4 IN d_6-DMSO.

Chemical shift (δ, ppm)	Multiplicity	Assignment (Fig.9)
7.92	doublets (J = 9 Hz)	2', 6'
6.93	doublets (J = 9 Hz)	3', 5'
6.79	singlet	8
6.48	singlet	3
6.20	singlet	6

The proton absorption of the hydroxyl groups is shifted to lower field (δ 10.2) due to intermolecular hydrogen bonding.

The H-3 signal with no proton neigbours appeared as a sharp singlet at δ 6.48.

The H-6 and H-8 protons are meta to each other. Hence, meta coupling is expected (J = 2.5 Hz). This was not observed in the spectra.

Protons at C-2', 3', 5' and 6 ', due to free rotation of the B-ring, appeared as two pairs of ortho coupled doublets.

H-2′ and H-3′ protons are ortho related, and appeared as a doublet (J = 9 Hz). The same is true for H-5′ and H-6′ protons. The H-3′, 5′ doublet occurred upfield from the H-2′, 6′ doublet due to the shielding effect of the hydroxyl substituent and deshielding influence of C-ring functions on H-2′ and H-6′.

H-2′ and H-6′ protons are identical to each other; hence, their signals are superimposed at δ 7.92. Similarly, due to the identical nature of H-3′ and H-5′; they are shown as a single signal at δ 6.93. The numbering system for apeginin is shown in figure 90 (Harborne, 1975; Markham, 1982). See appendix III for the NMR spectrum.

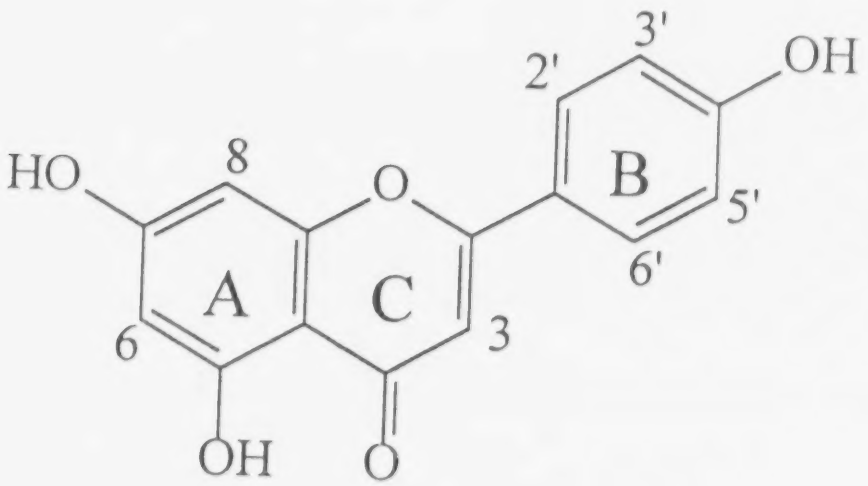


Figure 9. Structure of apigenin

4.4. Identification of compound # 3

Compound # 3 was identified as scopoletin based on its UV, NMR and MS characteristics and melting point.

4.4.1. Ultraviolet absorption spectroscopy analysis

Coumarins show characteristic absorption bands at 274 nm and 311 nm (log ϵ 4.03 and 3.72) (Murray, 1982). The introduction of a hydroxyl group into the coumarin nucleus causes a bathochromic shift of the principal absorption bands. The positions of the new maxima depends on the ability of the hydroxyl group to conjugate with the chromophoric group.

The characteristic absorption bands of compound # 3 were 243 (log ϵ =4.23), 259.3 (log ϵ =4.21), 269.5 (log ϵ =4.34), 306.2 (logϵ =4.18) and 348.5 (logϵ =4.08).

These bands arise from the $\pi \rightarrow \pi^*$ transition and $n \rightarrow \sigma^*$ transition, the latter being attributed to the oxygen atoms present in the molecule. The $n \rightarrow \pi^*$ transitions are represented by the longer wavelength bands than the $\pi \rightarrow \pi^*$ transitions since they require less energy for their transitions.

A marked bathochromic shift was observed for compound #3, when a sodium hydroxide solution was added. The shift from 348.5 to 395.2 nm is an indication of a 7-hydroxy-6-methoxy substitution. According to Murray (1982), the expected transtion was from 344 nm to 400 nm. See appendix I

for the UV spectrum.

4.4.2 Mass spectroscopic analysis of compound # 3

The high resolution mass spectroscopy of compound # 3 gave six relatively strong peaks (Table 14). The pattern of fragmentation for this compound is shown on figure 10.

The base peak with an intensity of 100% is the molecular ion. Its initial fragmentation gave an intense [M-15] ion due to loss of a methyl radical from the C-6 methoxyl group at m/z 177. This ion possesses a favourable para-quinonoid system for further fragmentation; subsequent loss of carbon monoxide gave rise to the m/z 149 ion.

Another fragmentation pattern of the molecular ion initially produced an [M-28] ion with relatively weak intensity at m/z 164. Loss of a methyl group from this fragment resulted in the formation m/z 149. This in turn produced the m/z 121 ion by losing another carbon monoxide group.

Further fragmentation of the m/z 121 ion could proceed via a substituted tropylium radical formed by rearrangement during the fragmentation process. The result is a 7 membered ring system which is capable of lossing two ethylenic groups to produce m/z 69 (Johnston, 1966; Murray, 1982). See appendix II for the mass spectrum.

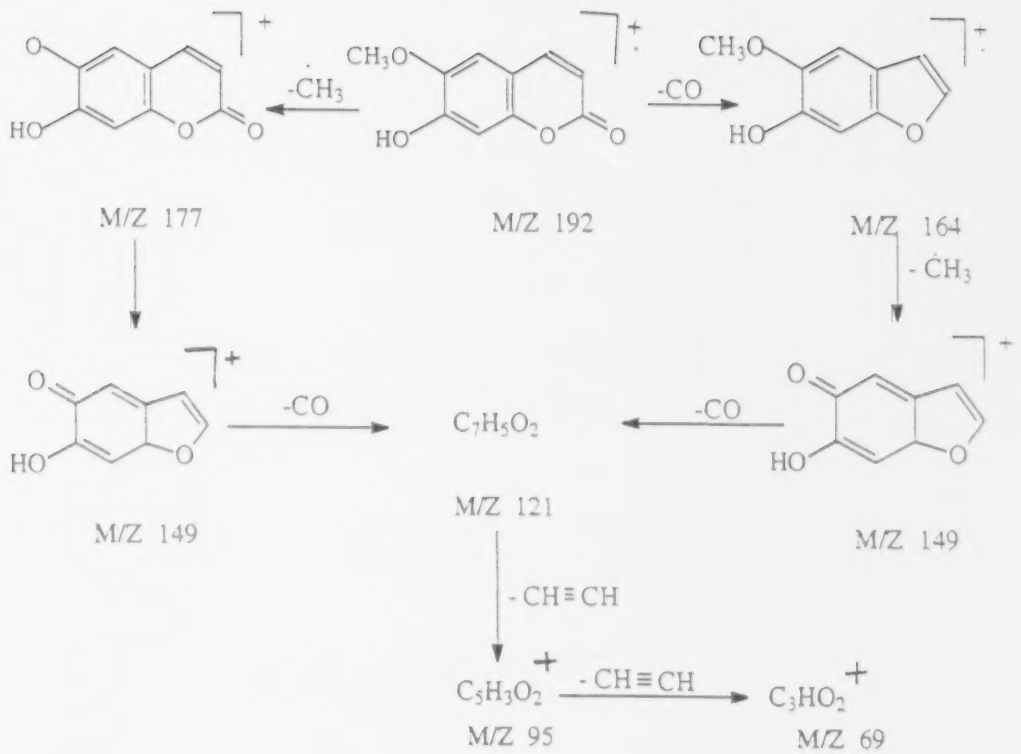


Figure 10. Mass fragmentation pattern of compound # 3

Table 14. Relative abundance of some important fragment ions of compound # 3

m/z	% relative abundance	Formula
192	100	$C_{10}H_8O_4$
177	55.62	$C_9H_5O_4$
164	20.35	$C_9H_8O_3$
149	41.49	C_8H_5O3
121	13.46	$C_7H_5O_2$
69	56.39	C_4H_5O

4.4.3. Nuclear magnetic resonance spectroscopy of compound #3

THE ^1H NMR signals of compound #3 were assigned as shown in table 15.

Table 15. ^1H NMR Chemical shift for compound # 3 in d_6- DMSO

Chemical shift (δ,ppm)	Multiplicity	Assignment (Figure 11)
7.72	doublet	H-4
6.92	singlet	H-5
6.40	singlet	H-8
5.90	doublet	H-3

All coumarins unsubstituted in the pyrone ring exhibit doublets arising from the cis protons H-3 and H-4 of this

ring (Figure 11). According to Steck (1972), this feature may be taken as a firm indication of the presence of a coumarin nucleus.

The ^1H NMR of compound #3 showed two doublets at δ 7.72 and δ 5.9 (J=9.5 Hz). These could be assigned to H-4 and H-3 respectively, since the hydrogen on C-3 is more shielded than the one on C-4. Therefore H-3 will be observed at higher field than H-4.

H-5 resonates at δ 6.92 and appears as a singlet. This shows the absence of protons in the neighbourhood of C-5. The same is true for H-8, but because it is more shielded by the oxygen atom at C-7 it is expected to appear at higher field (δ 6.40) relative to H-5.

The protons of the methoxy groups were expected to appear at around δ 3.80. This was not observed, possibly due to the masking effect of the broad peak of water, which is present due to the fact that d_6-DMSO absorbs moisture on standing.

The proton of the hydroxyl group at C-7 was also expected to be very far downfield, but was not observed.

The different signals from δ0 to δ 3 and the one at δ 6.8 could have been from either impurities and/or the solvent d_6-DMSO (DMSO-d_6 is the solvent recommended in literatures for NMR spectral analysis of such compounds). See appendix III for the NMR spectrum.

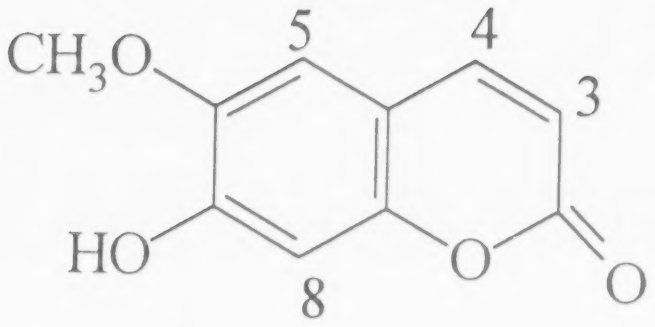


Figure 11. scopoletin

4.5. Identification of compounds # 1, 2 and 5

Compounds # 1, 2, and 5 were not satisfactorily identified due to lack of sufficient sample for spectroscopic studies and degradation. The information gathered and the limited data obtained for these compounds are discussed below.

4.5.1. Ultraviolet spectroscopy analysis of compounds #1, 2 and 5

The UV absorption peaks for compounds # 1, and 5, measured in methanol, are given in tables 16 and 17 respectively. The absorption maxima for compound # 2 was not measured due to the very small amount of the compound isolated.

Table 16. UV absorption peaks (nm) for compound # 1

"MeOH" spectrum	"NaOH" spectrum
204.3	205.9
265.2	272.1
338	385

A bathochromic shift of 50 nm was observed in the "NaOH" spec rum of compound # 1 compared to the "MeOH" one. This is a clear indication of the presence of a free hydroxyl in the molecule. If the compound is assumed to be a coumarin derivative (an assumption based on TLC colour

reaction, melting point, and mass spectral data), then the hydroxyl group will be in position 4, 5, or 7. This is derived from the fact that electron delocalization of the phenoxide ion with the pyrone-carbonyl group is possible for salts of 4-, 5-, and 7- hydroxycoumarins (Murray, 1982).

The "$AlCl_3$" spectrum, on the other hand has shown no change in the absorption maxima of compound # 1. Therefore it is concluded that neither neighbouring hydroxyl groups nor hydroxy-keto groups are present (Markham, 1982). See appendix I for the UV spectrum.

Table 17. UV absorption peaks (nm) for compound # 5

"MeOH" spectrum	"NaOH" spectrum	"$AlCl_3$" spectrum
205	205.9	203.5
242	243.4	230.1
258	258.2	258.5
268.9	268.9	269.3
306.5	-----	306.7
325.6	325.7	326.1
347.8	396.5	347.7

The "NaOH" spectrum of compound # 5 shows a bathochromic shift of 50 nm compared to that of the "MeOH" spectrum. Therefore, the presence of a hydroxyl group can be suggested. The "$AlCl_3$" spectrum also showed a marked bathochromic shift of 50 nm. As mentioned earlier this is a good indication of the presence of either neighbouring hydroxyl groups or a hydroxy-keto group in the molecule.

4.5.2. MASS SPECTROSCOPY ANALYSIS OF COMPOUND # 1, 2, and 5

The high resolution mass spectra of compound # 1 and 5 each gave eight prominent fragment ions. The relative abundance of these ions is given in table 18 (compound # 1) and 19 (compound # 5). The mass spectrum of compound # 2 was not obtained.

Table 18. Relative abundance of some important fragment ions of compound # 1

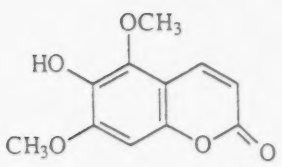
M/Z	% Relative abundance	Formula
222	100	$C_{11}H_{10}O_5$
207	26.35	$C_{10}H_7O_5$
194	10.93	$C_{10}H_{10}O_4$
179	12.81	$C_9H_7O_4$
141	12.27	$C_8H_7O_3$

Based on the TLC colour reaction and UV absorption spectrum and assuming that the base peak at m/z 222 is the molecular ion, the fragmentation pattern of compound # 1 is considered to be consistant with that of a coumarin with one hydroxyl and two methoxyl substituents. Such a coumarin derivative can exist in one of the eight isomeric forms shown on figure 13. The melting point of the compound (185-187.5^0 C) however is similar to that of tomentin (5-hydroxy-

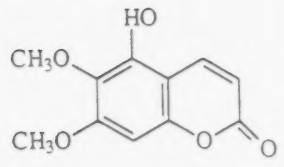
6,7-dimethoxycoumarin), 185^{0} C (murray, 1982). Therefore compound # 1 may be tomentin and its fragmentation pattern, which basically involves loss of carbon monoxide and methyl groups, as in the case of compound # 3 is shown on figure 13 (Johnston, 1966, Murray,1982). See appendix II for the mass spectrum.

Table 19. Relative aboundance of some important fragment ions of compound # 5

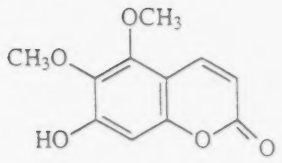
M/Z	% Relative abundance	Formula
279	13.76	$C_{16}H_{23}O_4$
256	2.93	$C_{16}H_{32}O_2$
236	2.57	no match
167	25.84	$C_8H_7O_4$
151	9.49	$C_{11}H_{19}$
149	72.49	$C_8H_5O_3$
111	14.29	$C_7H_{11}O$
83	33.29	C_5H_7O
60	100	$C_2H_4O_2$



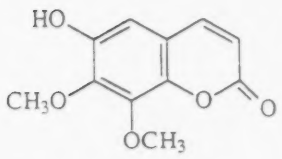
Fraxinol
171-173



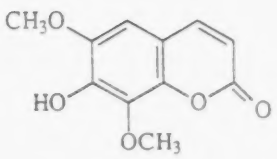
Tomentin
185



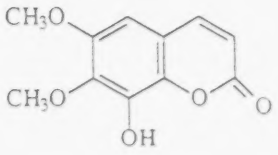
Umekalin
146-147



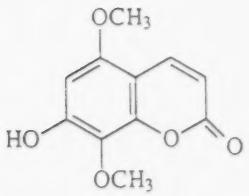
Arscotin
192-193



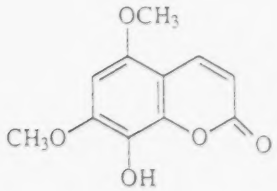
Isofraxidin
228-230



Fraxidin
196-197



7-Hydroxy-5,8-dimethoxycoumarin
197-199



Leptodactylon
149-152

Figure 12. Structures and reported Melting points of hydroxy-dimethoxycoumarins (Murray, 1982)

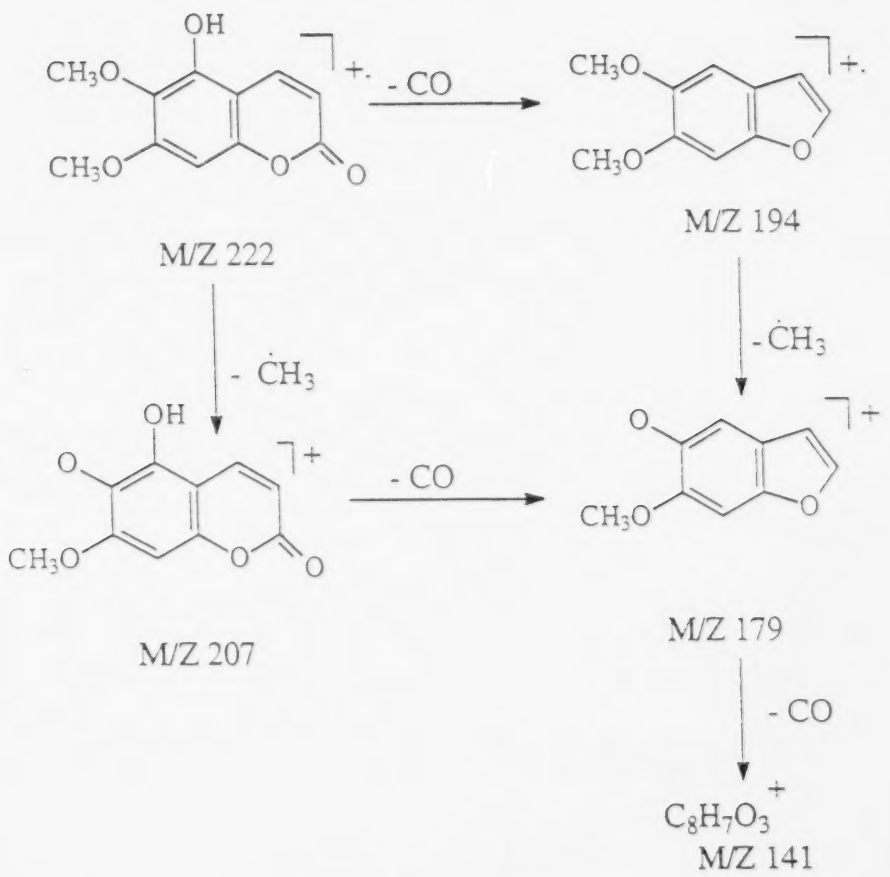


Figure 13. Fragmentation pattern of tomentin

4.5.3. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF COMPOUNDS # 1, 2 AND 5

The initial NMR spectra of these compounds were not clear enough for the purpose of chemical shift assignments. This could be due to the presence of impurities both in the samples and the solvent used. It was not possible to re-run the NMR spectroscopy since sufficient quantities of these compounds were not available.

CHAPTER VI

CONCLUSION

The extraction method with a mixture of methanol and water gave 0.26 percent amount of the crude extract of *M. martima*.

The solvent system used for TLC examination [ethyl acetate:methanol:water in the ratio of (100:16.5:13.5)] revealed the highest number of components compared to other solvent systems used. *M. martima* flower extract contains at least 15 different components.

Column chromatography, with a number of solvent systems, was tried to isolate individual components. However, column chromatography was found to be useful only for the initial stage of fractionation. Preparative TLC, though time consuming, was found to be a suitable means of isolating of the constituents of *M. martima*.

Due to the small amount of compounds # 1,2, and 5 isolated, and possible degradation on storage, the spectroscopic methods, particularly ^{1}H NMR failed to provide clean spectra useful for the identification of these compounds.

With the help of the experimental data obtained from

TLC, melting point, UV, ^{1}H NMR and high resolution MS, compounds # 3 and # 4 are satisfactorily identified.

For compound # 3, the match in melting point, the signals from ^{1}H NMR, the UV absorption bands in MeOH and NaOH, and the fragment ions from MS analysis provided sufficient data for its identification. By interpreting these data and comparing with the literature values, compound # 3 was identified as scopoletin (6-methoxy-7-hydroxycoumarin).

For compound # 4, co-TLC with authentic sample, ^{1}H NMR signals, UV absorption bands in ethanol, $AlCl_3$ and $AlCl_3$/HCl and fragment ions from high resolution MS were used to establish its identity. Interpretation of these data and comparison with literature values indicated that compound # 4 is apigenin (4',5,7-trihydroxy flavone).

From the TLC, melting point UV, and MS data obtained compound # 1 is probably tomentin (5- hydroxy-6,7-dimethoxycoumarin).

The two characterized compounds apigenin and scopoletin and the tentatively identified compound tomentin have been isolated from *M. martima* for the first time in this investigation. These results are in agreement with the

occurrence of flavonoids and coumarins in other Matricaria species.

For detailed chemical investigation of *M. martima* a large amount of the plant material is recommended.

CHAPTER VI

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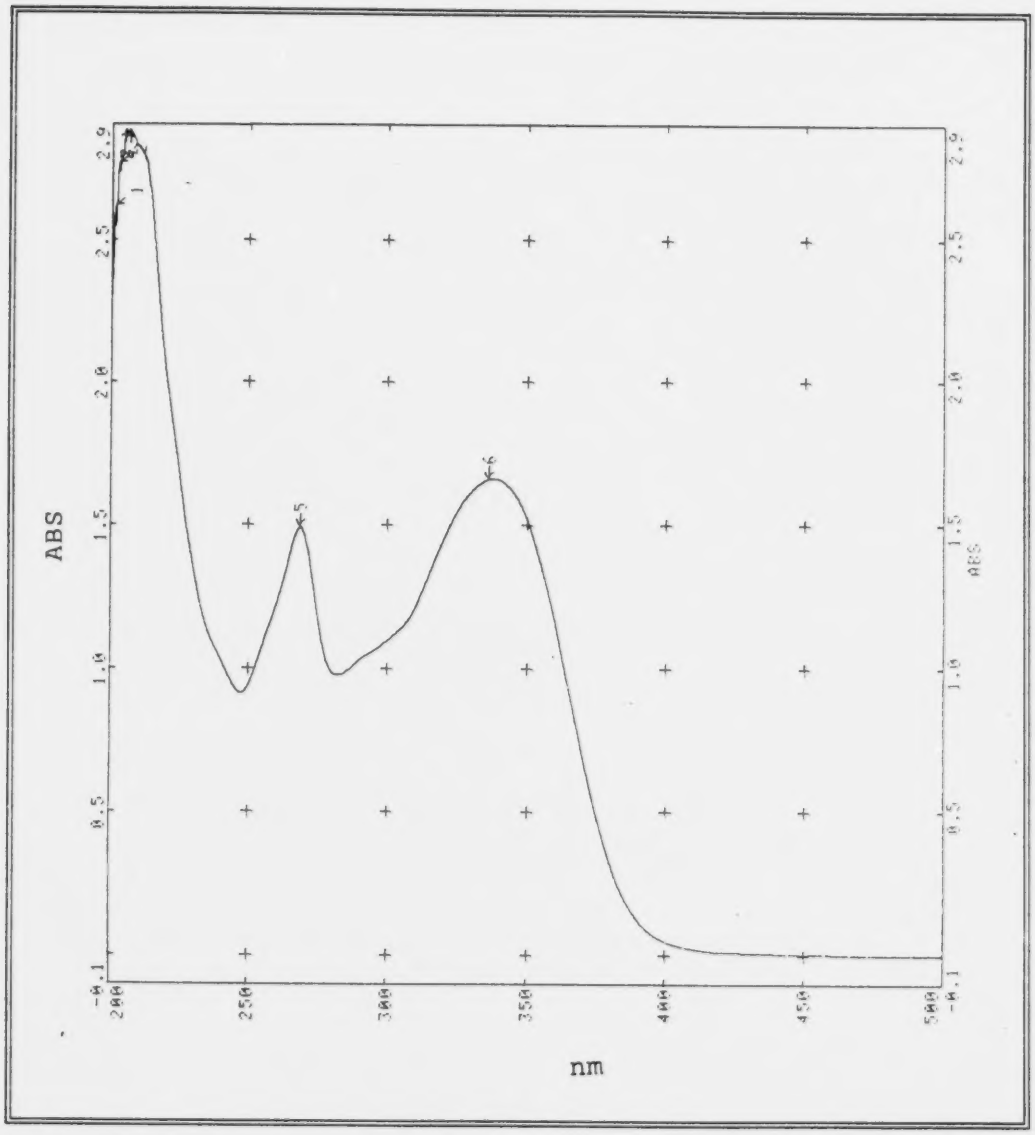
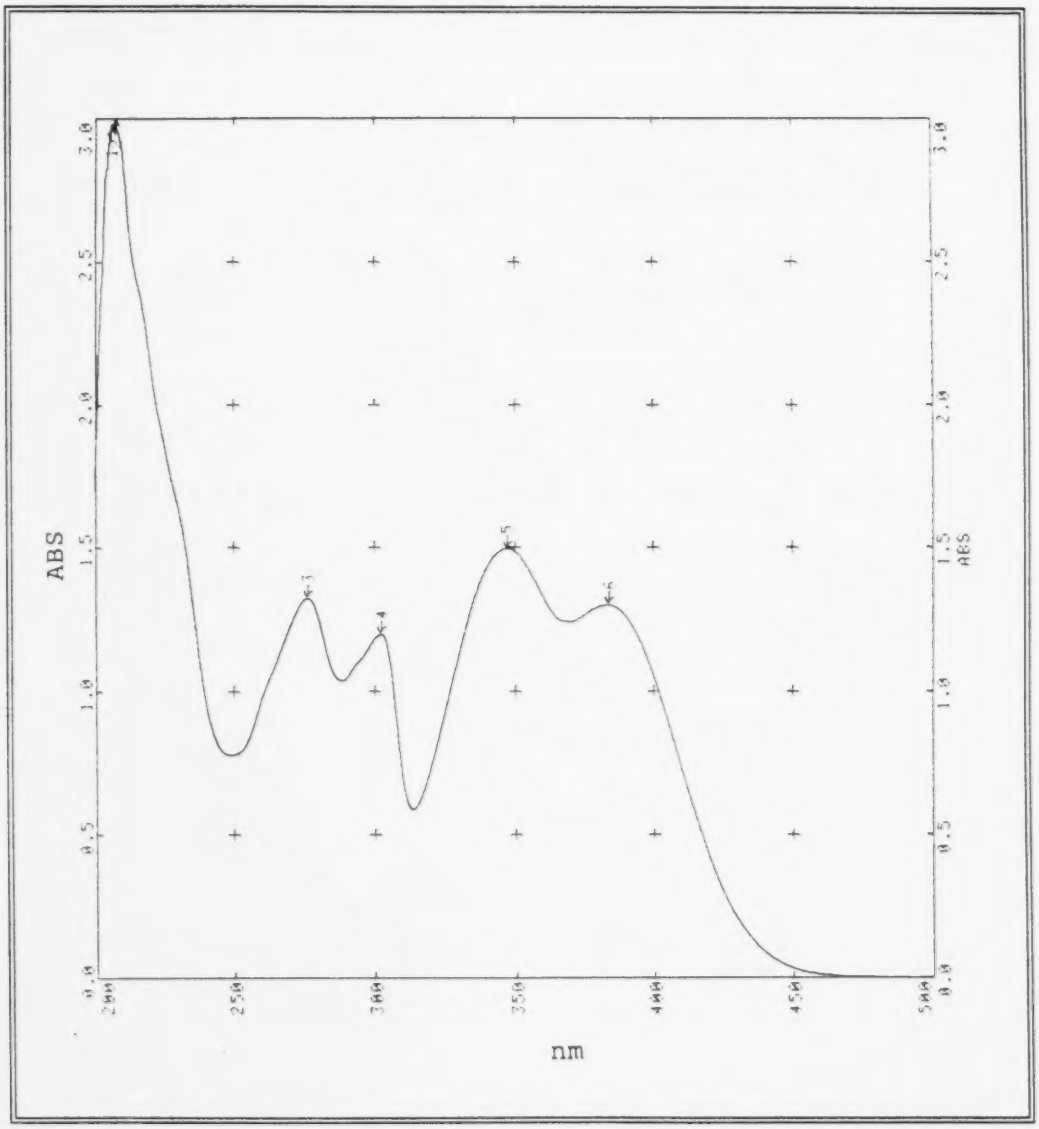
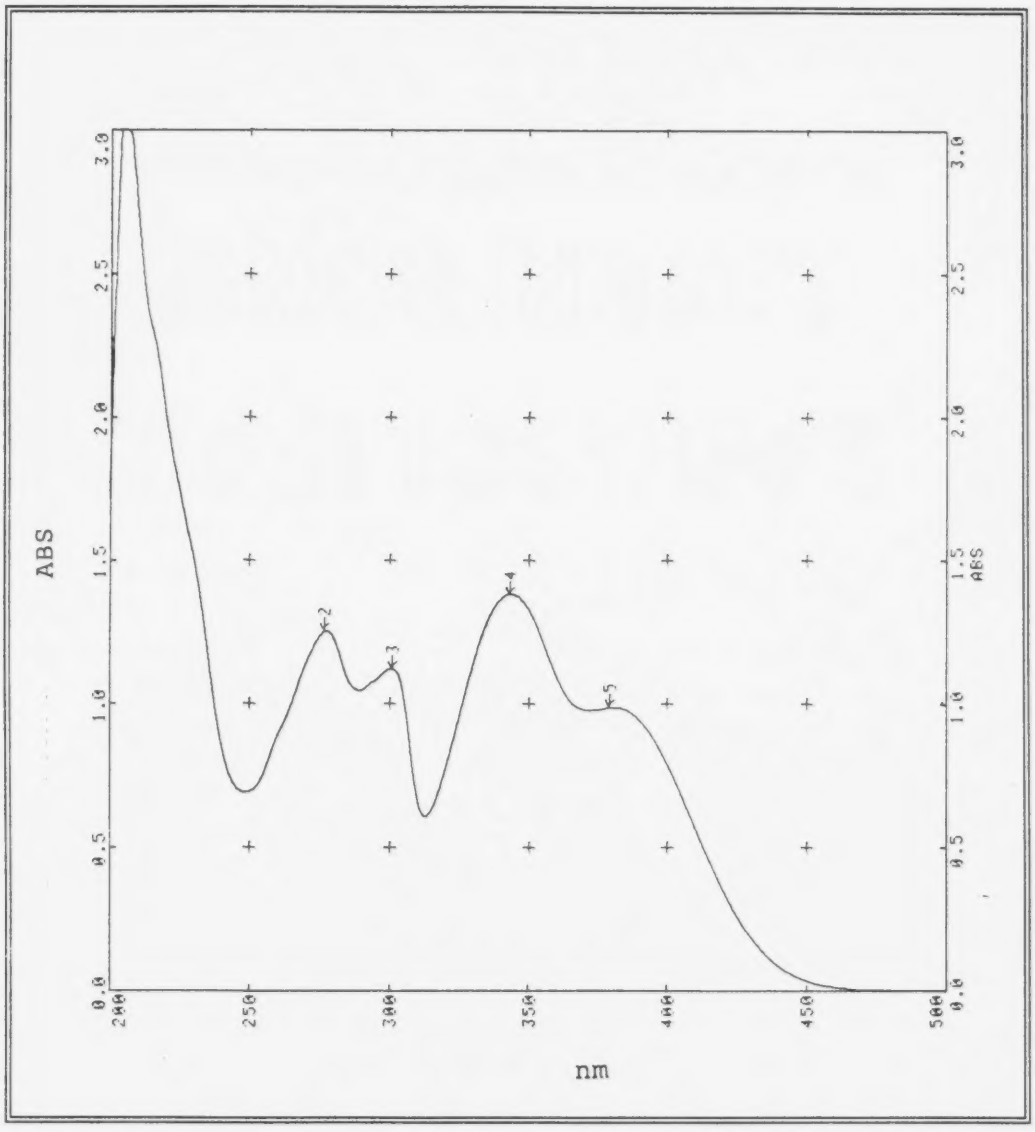
APPENDIX I. UV spectra of the isolated compounds



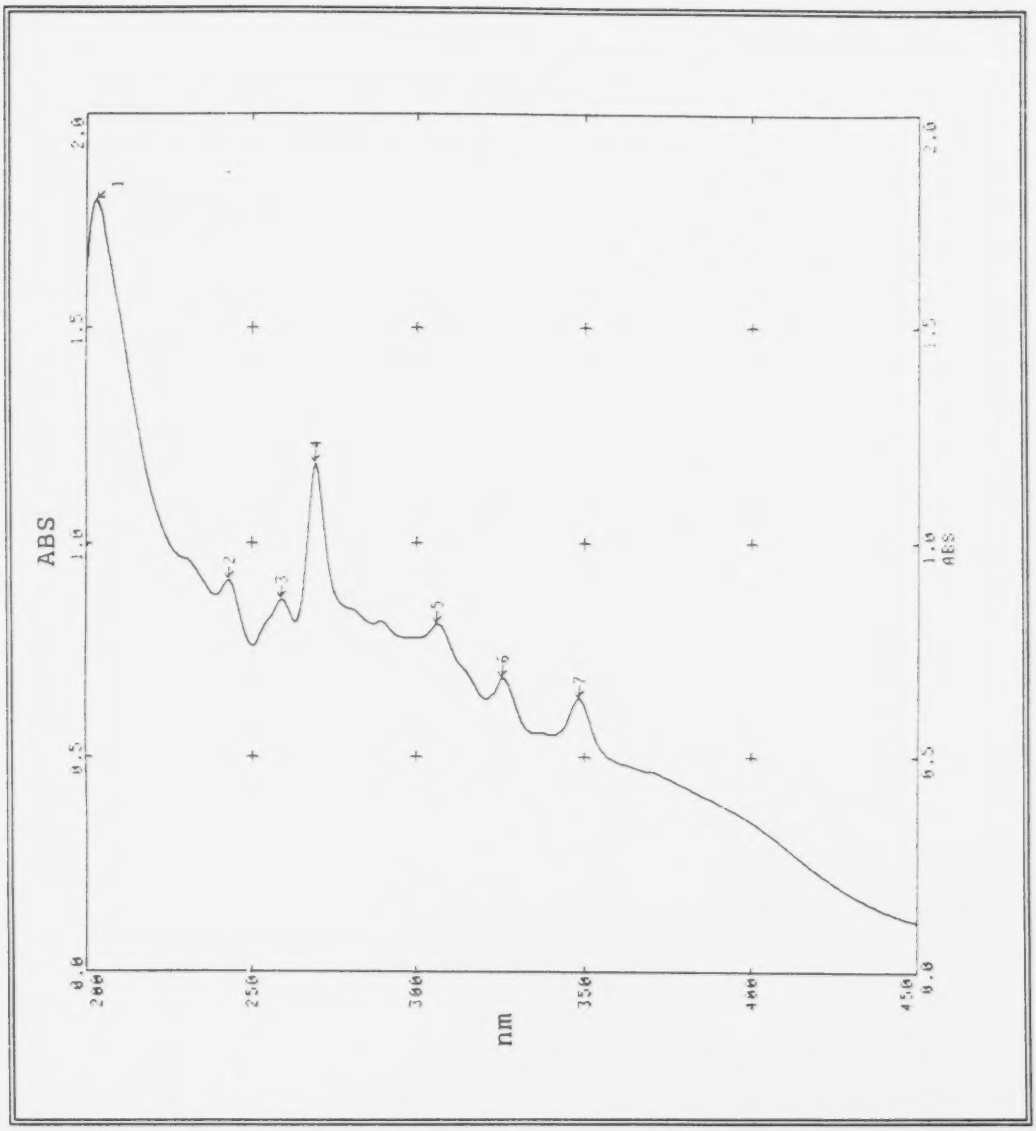
"MeOH" spectrum of compound # 4



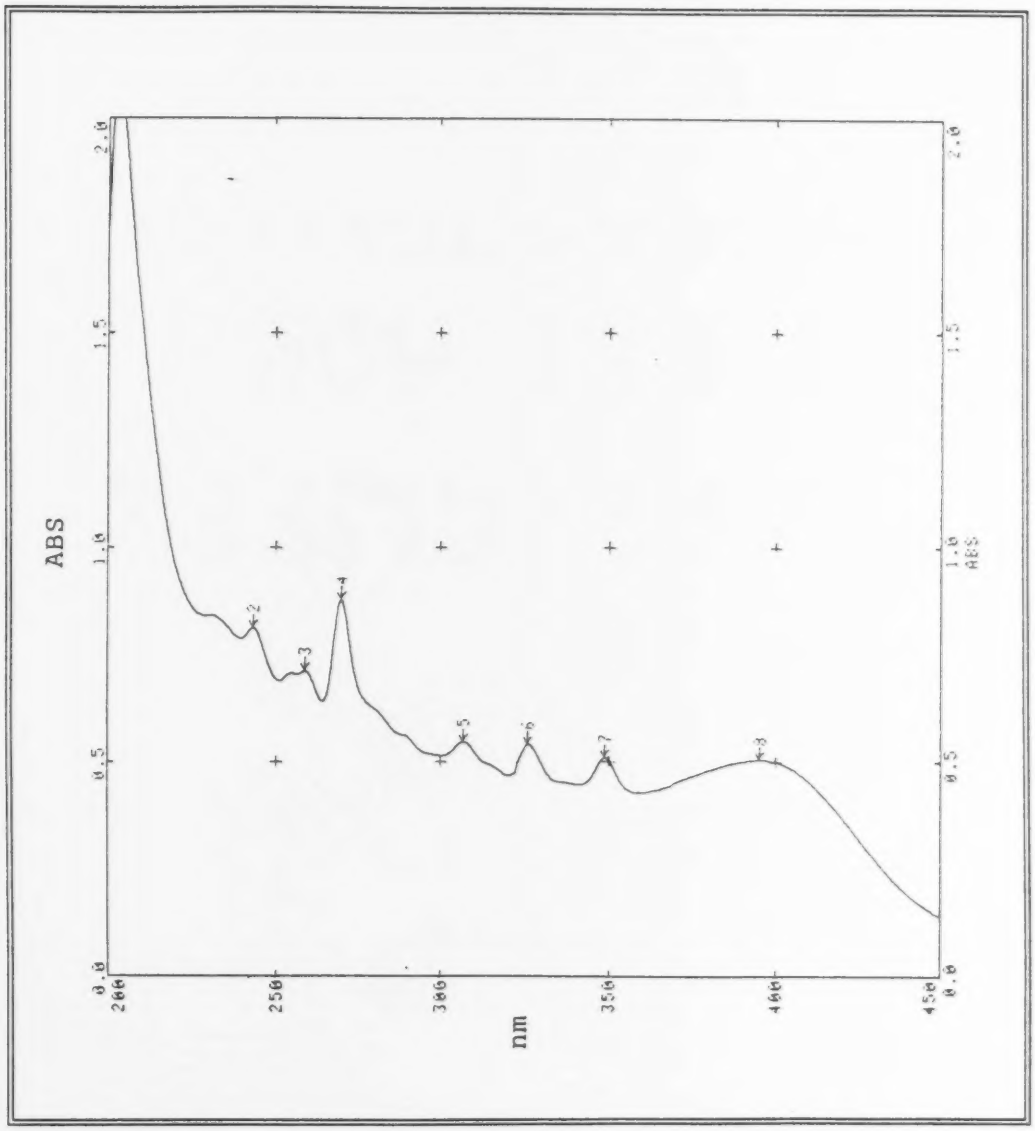
"$AlCl_3$" spectrum of compound # 4



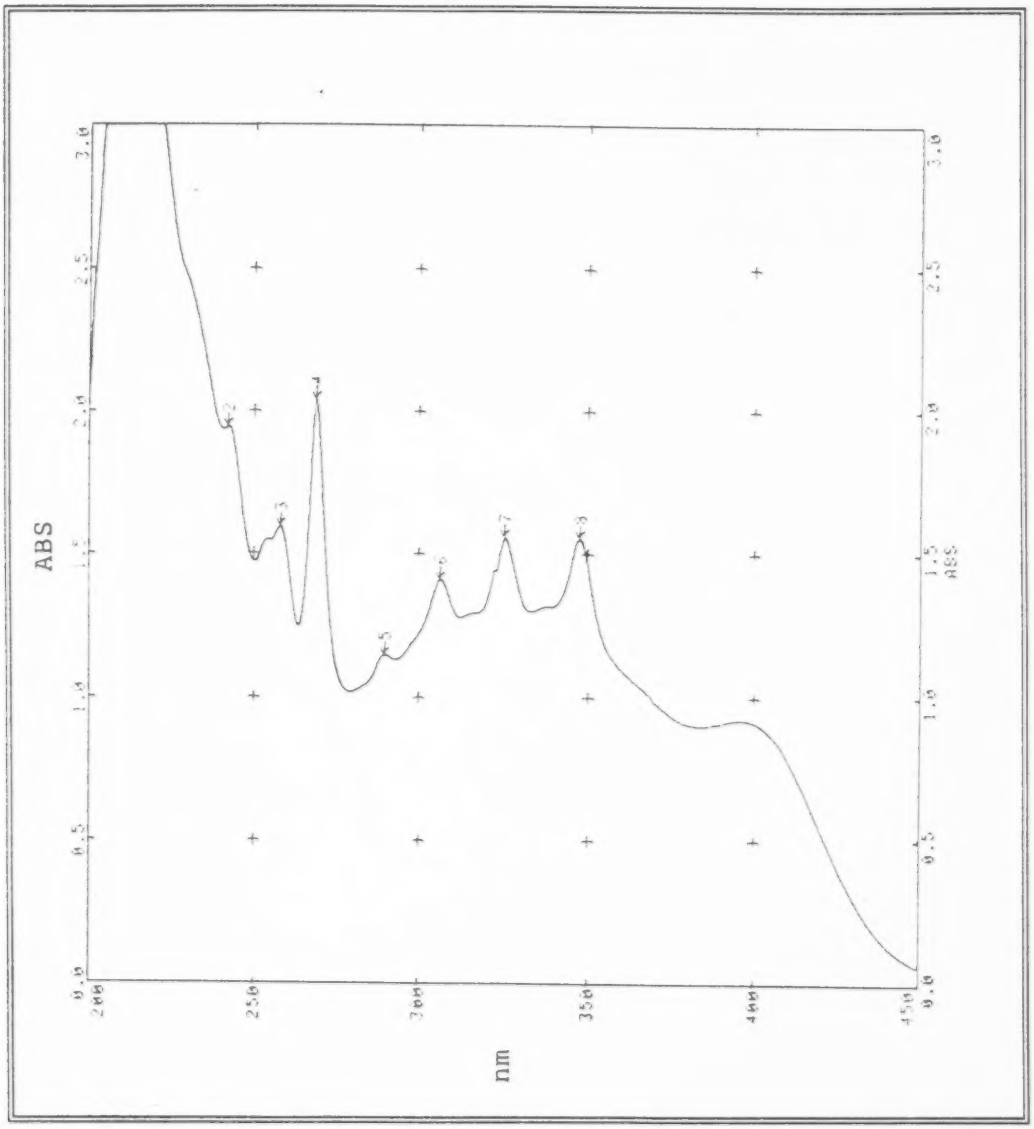
"$AlCl_3$/HCl" spectrum of compound # 4



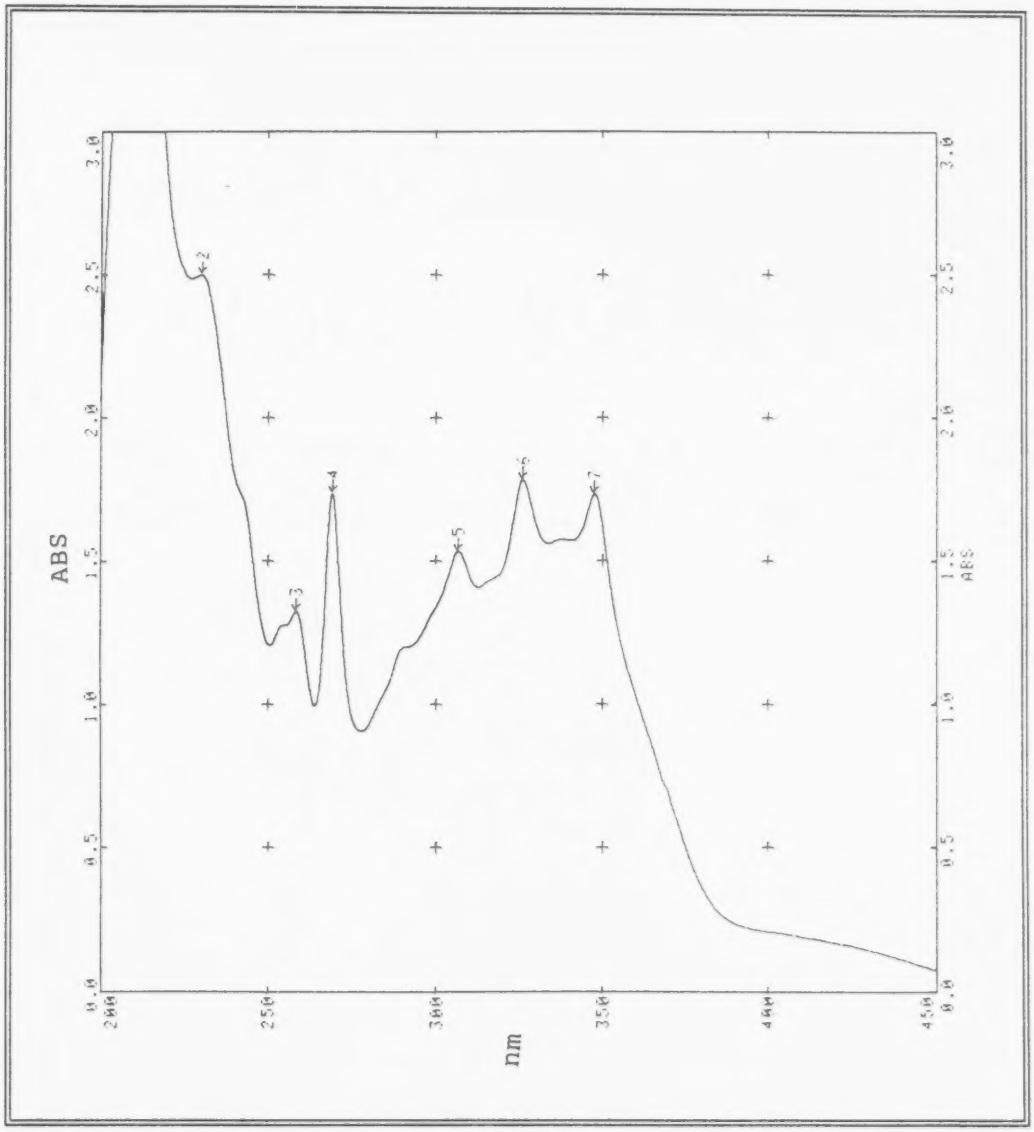
"MeOH" spectrum of compound # 3



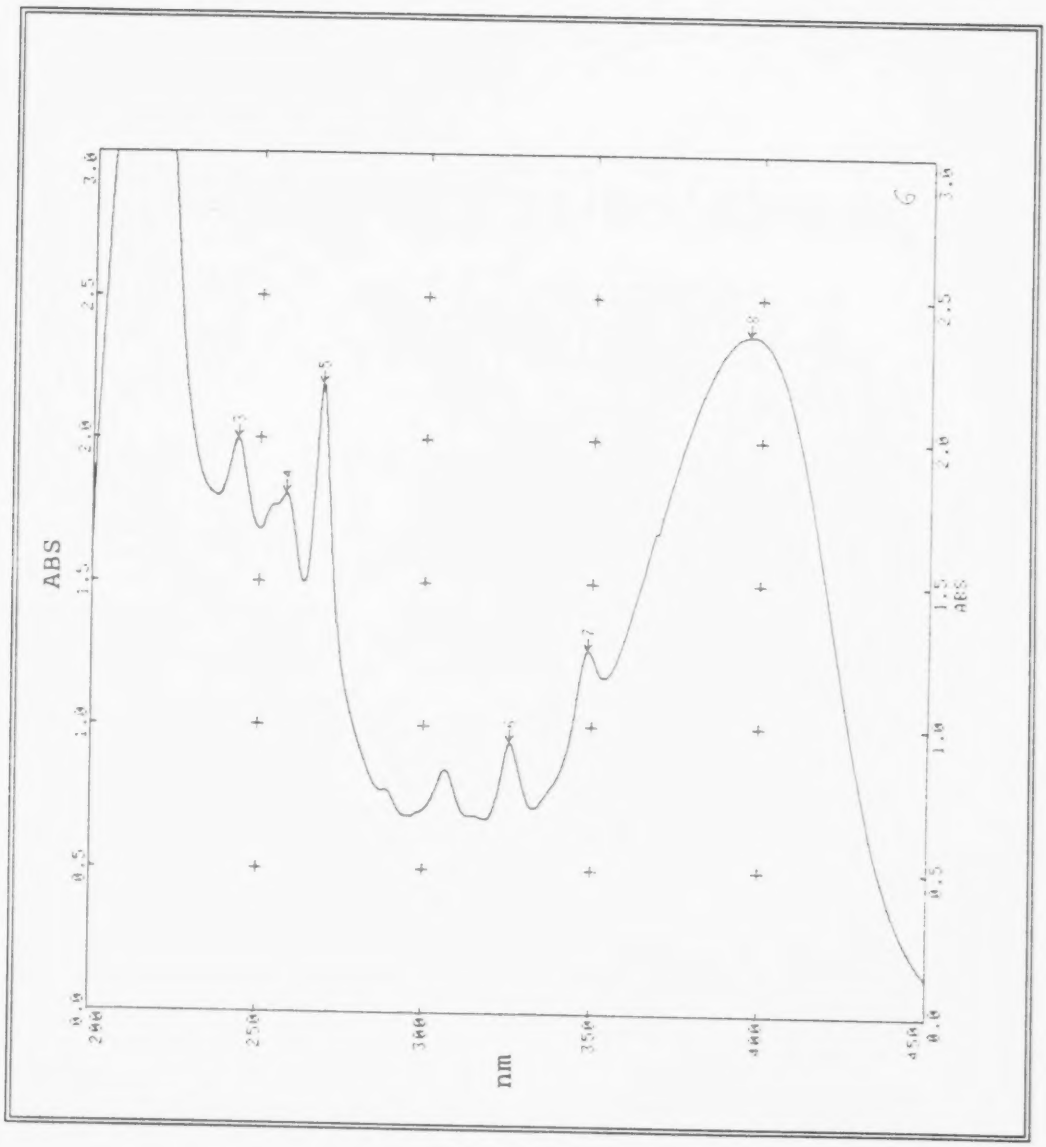
"NaOH" spectrum of compound # 3



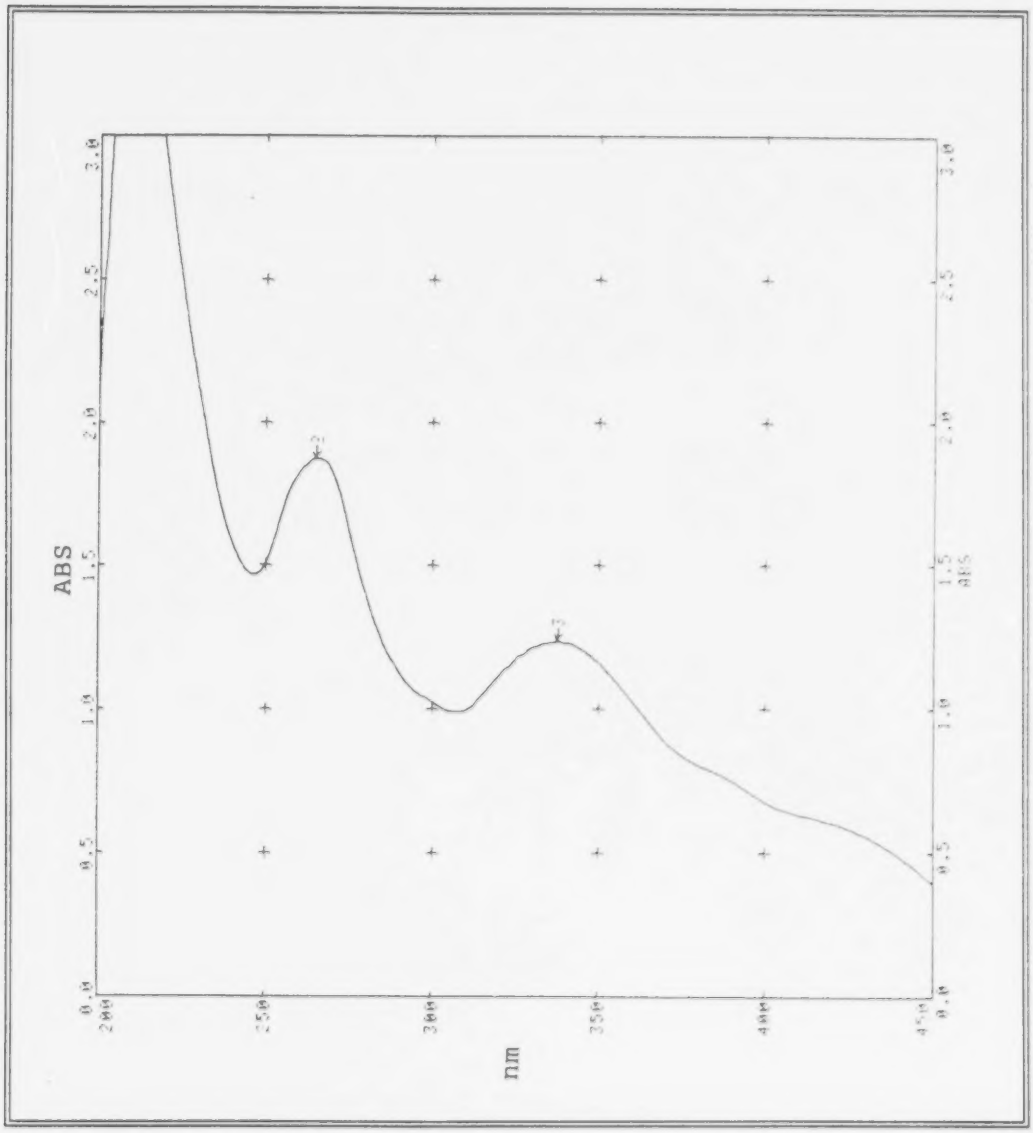
"MeOH" spectrum of compound # 1



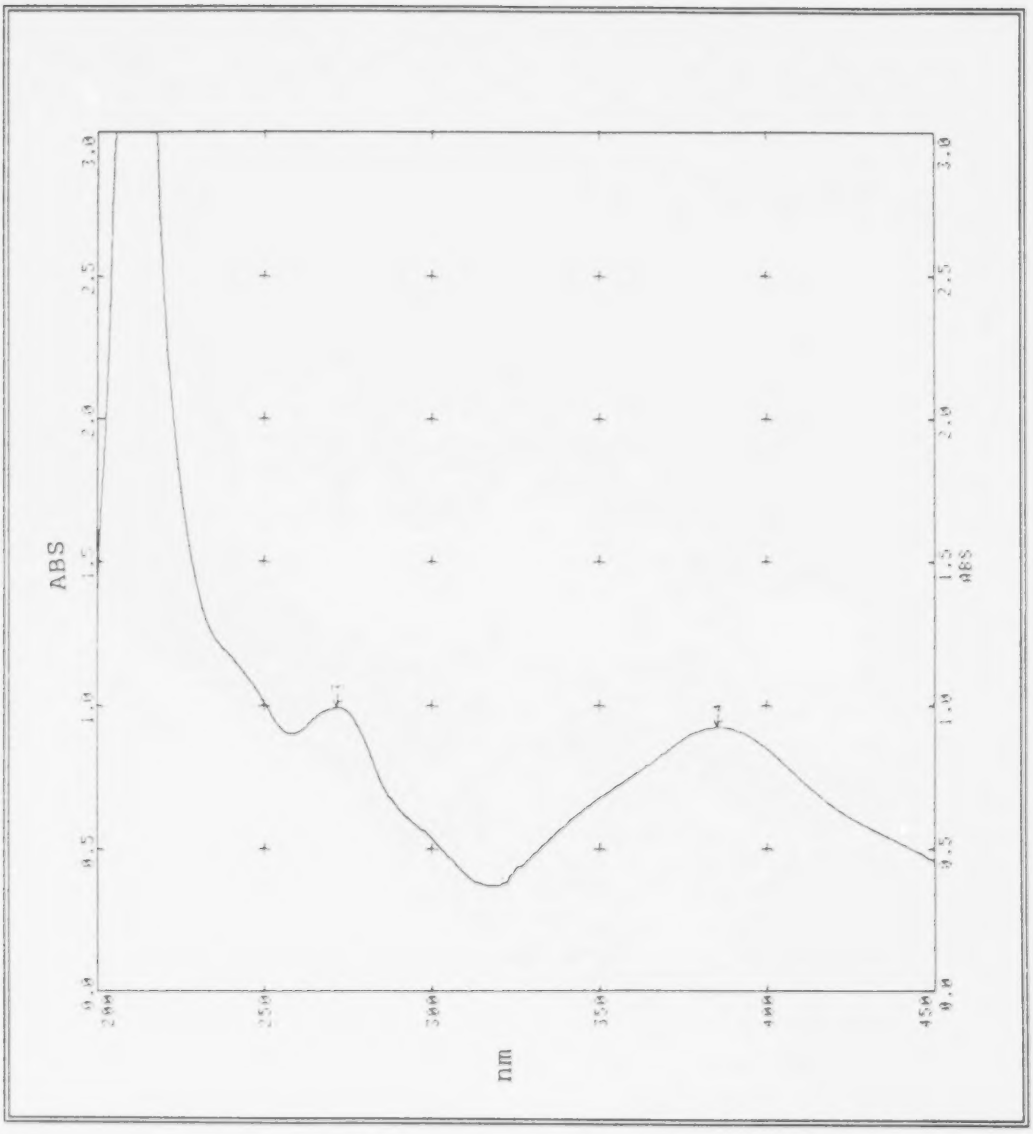
"$AlCl_3$" spectrum of compound # 1



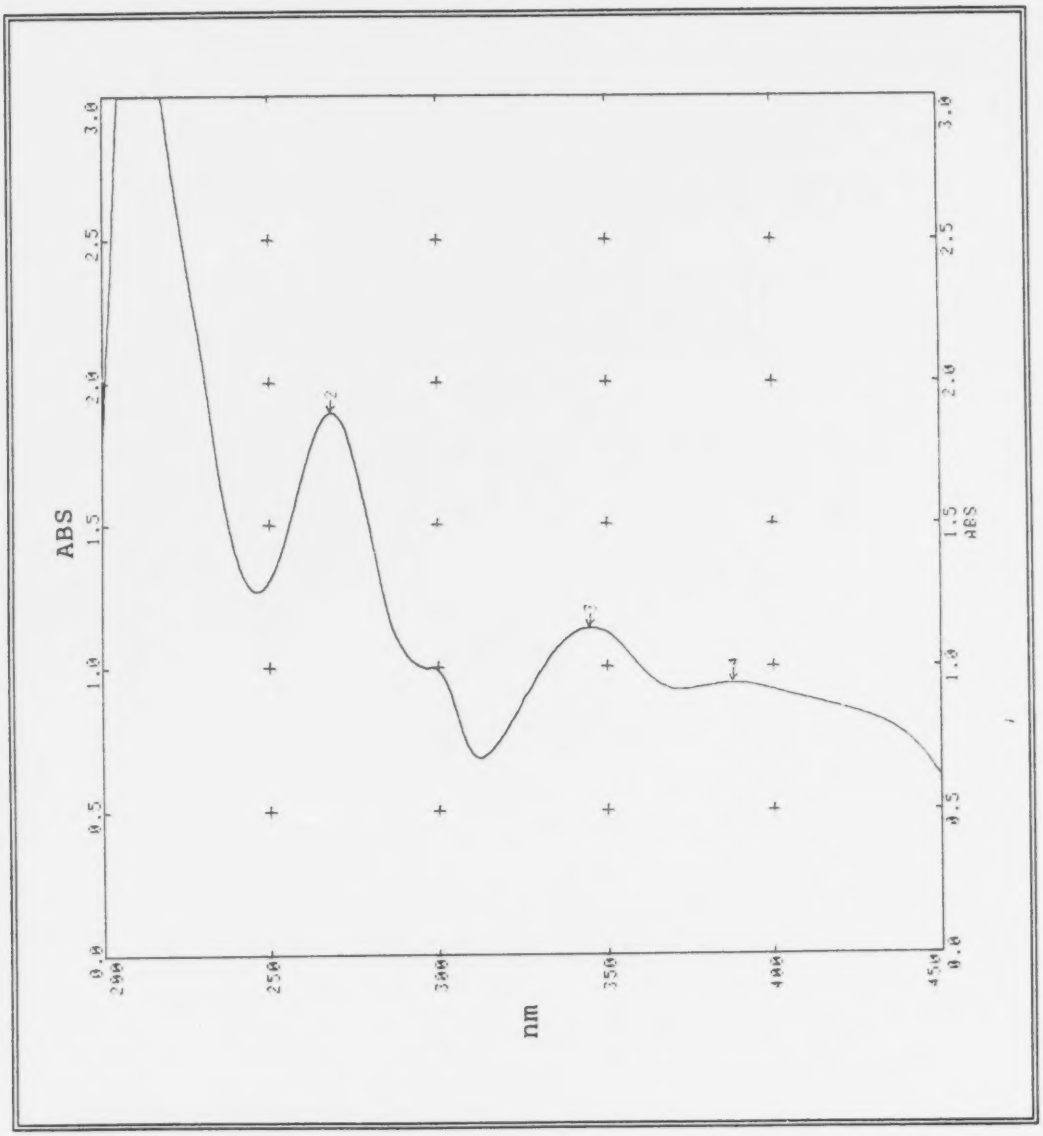
"NaOH" spectrum of compound # 1



"MeOH" spectrum of compound # 5

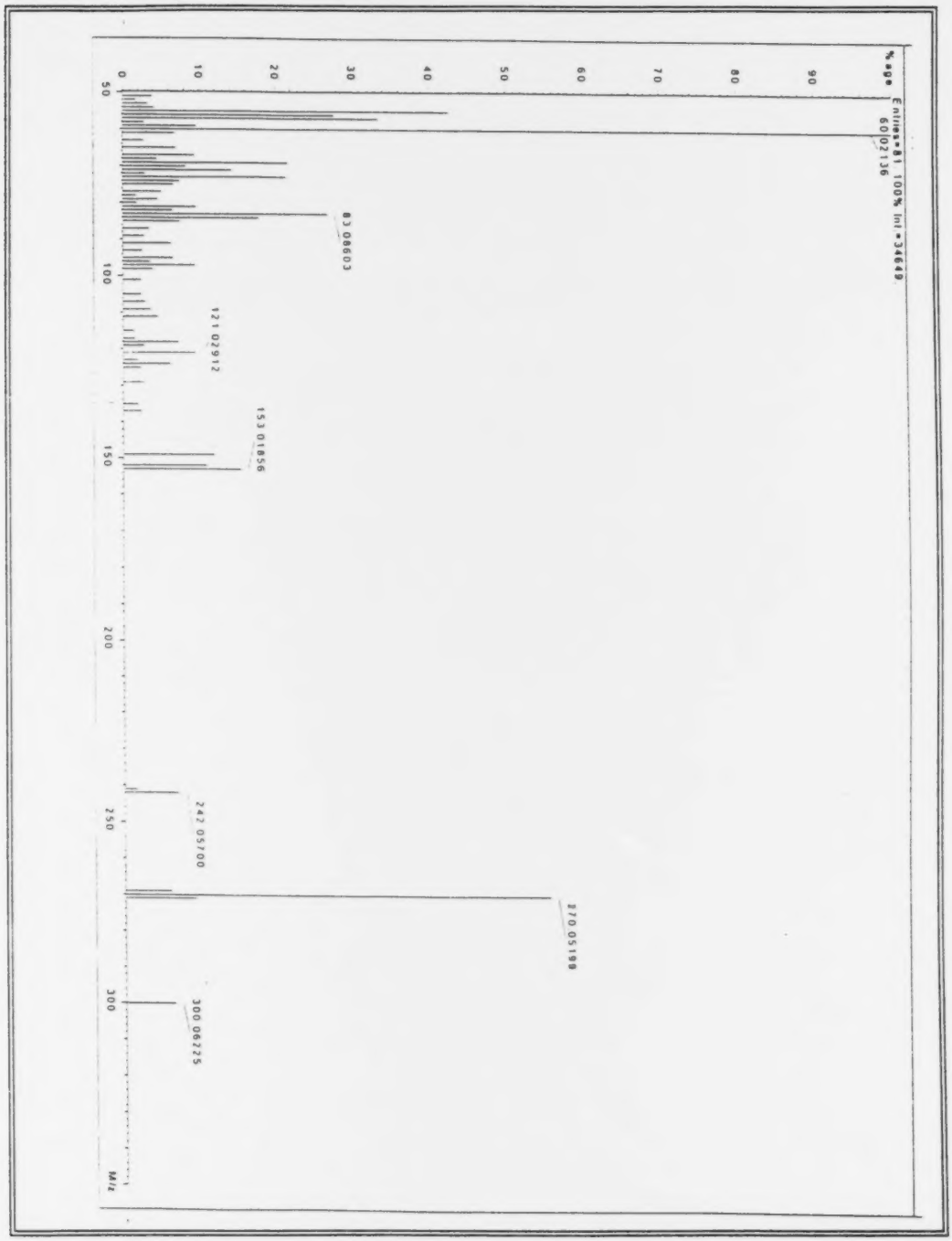


"$AlCl_3$" spectrum of compound # 5

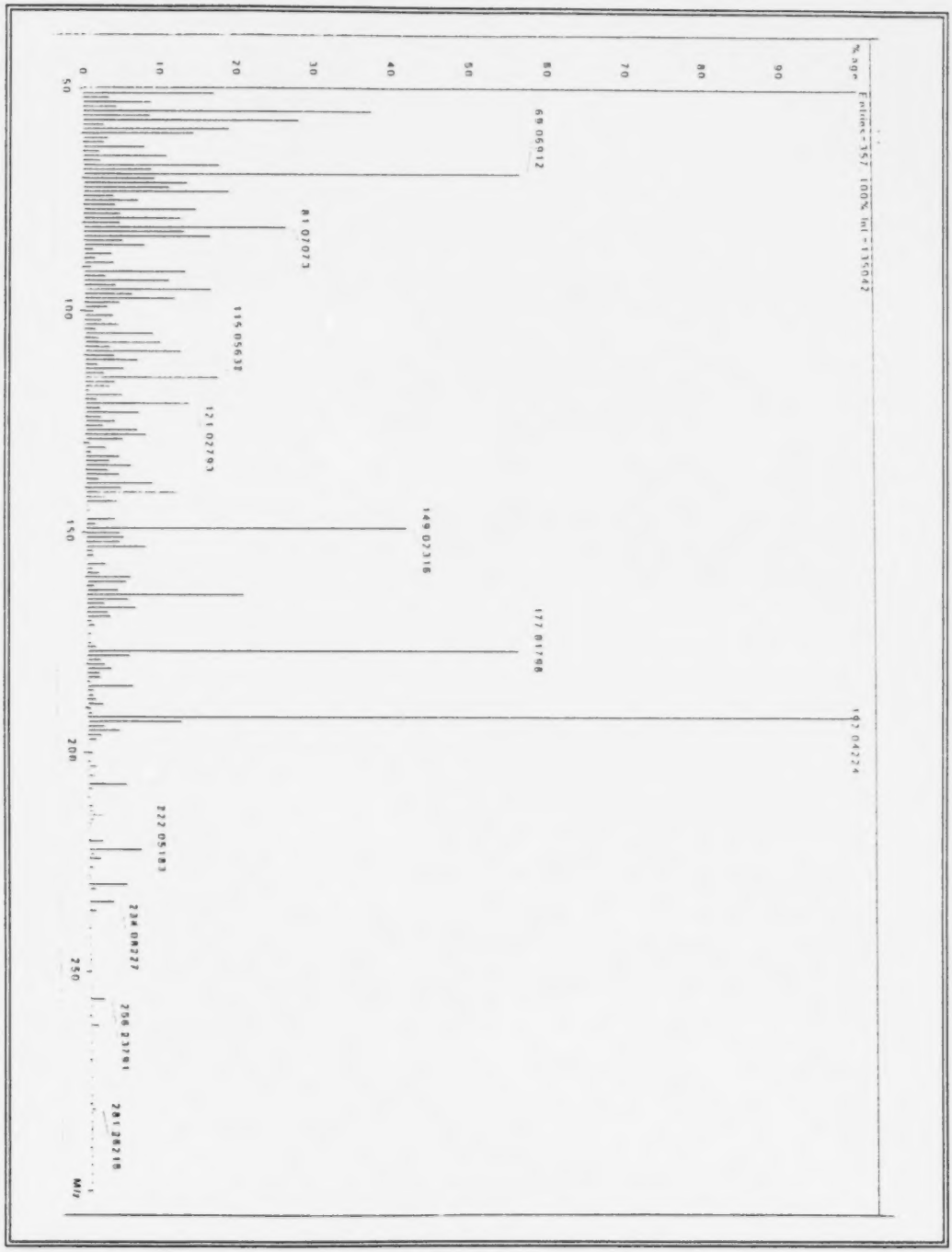


"NaOH" spectrum of compound # 5

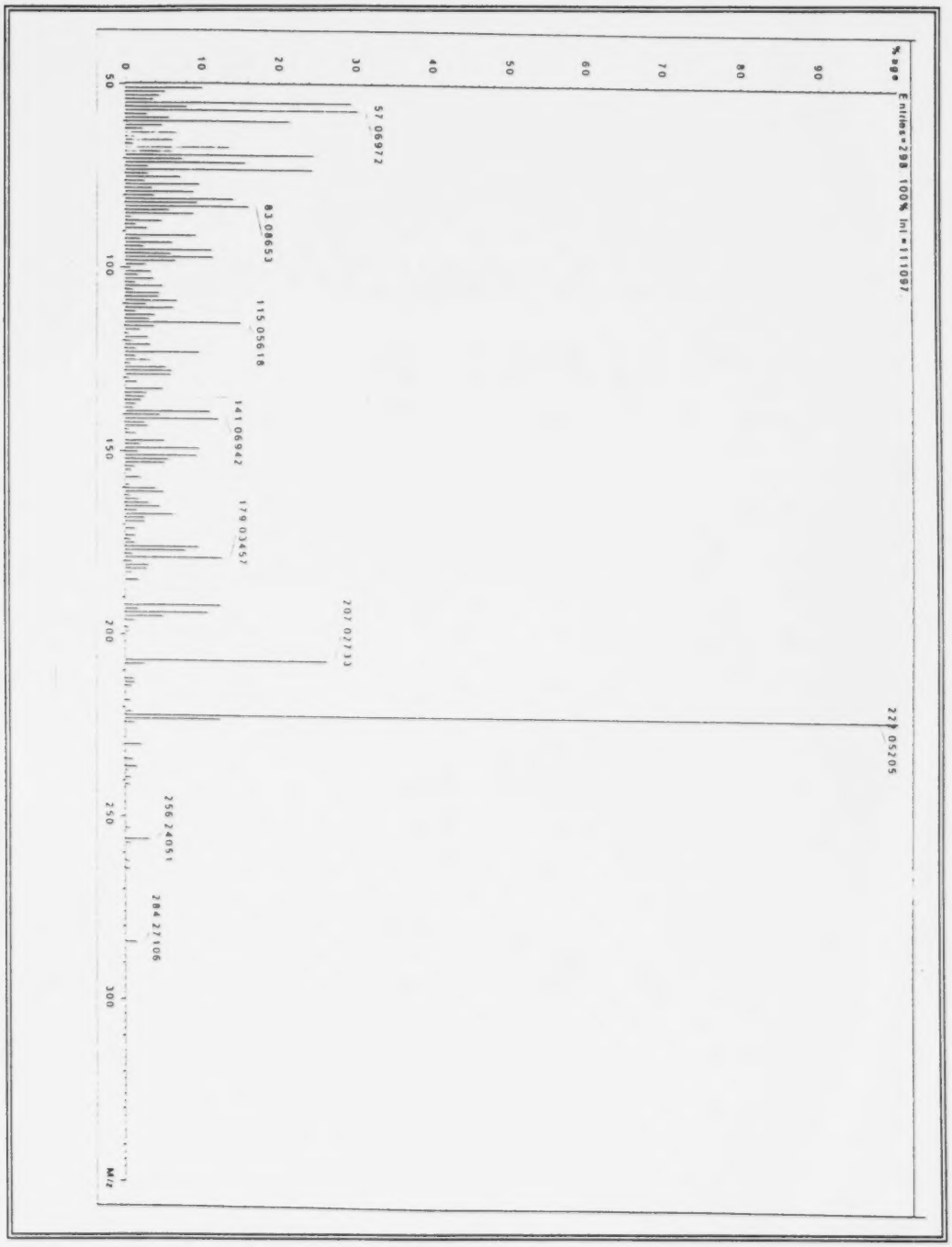
APPENDIX II. Mass spectra of the isolated compounds



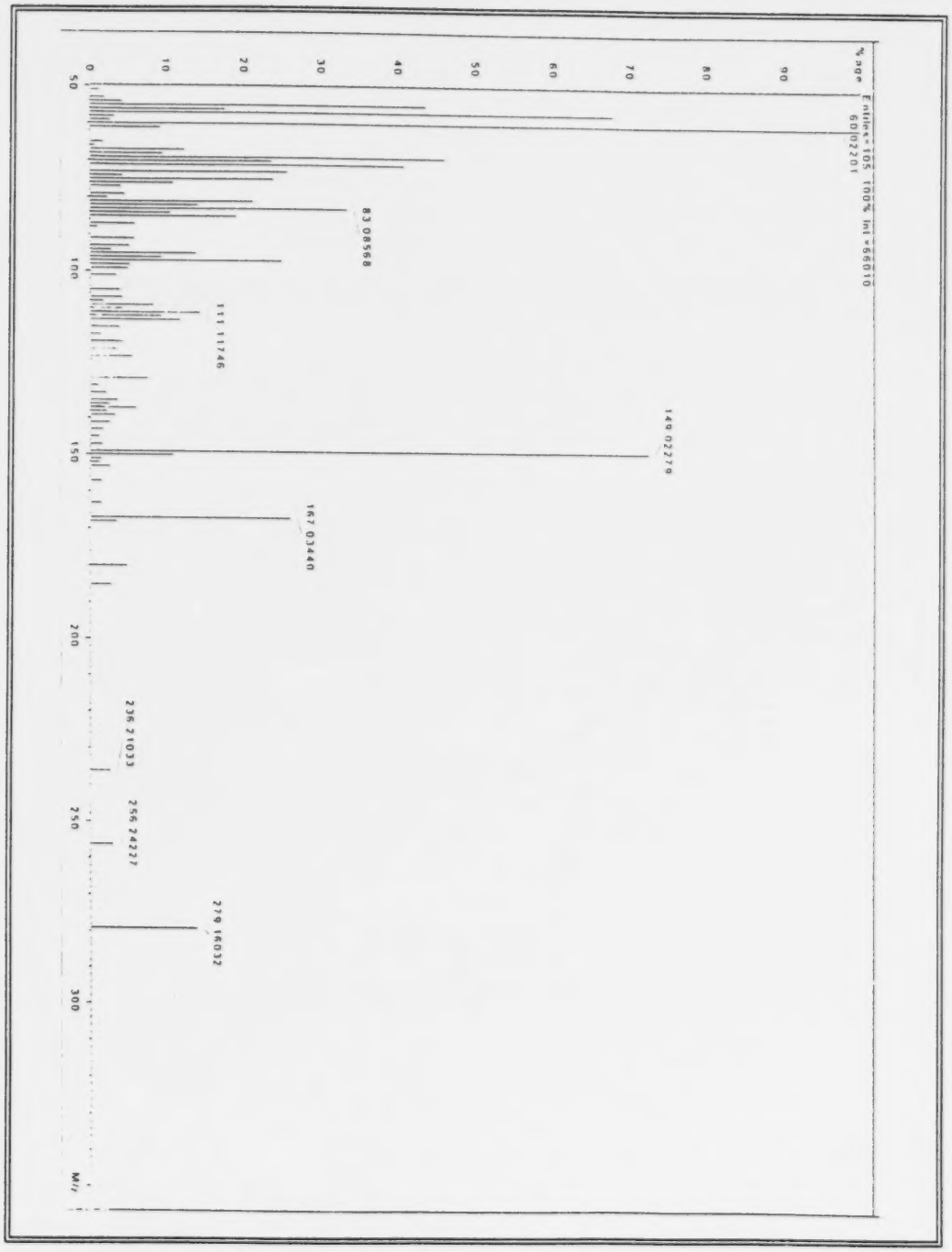
Mass spectrum of compound # 4



Mass spectrum of compound # 3

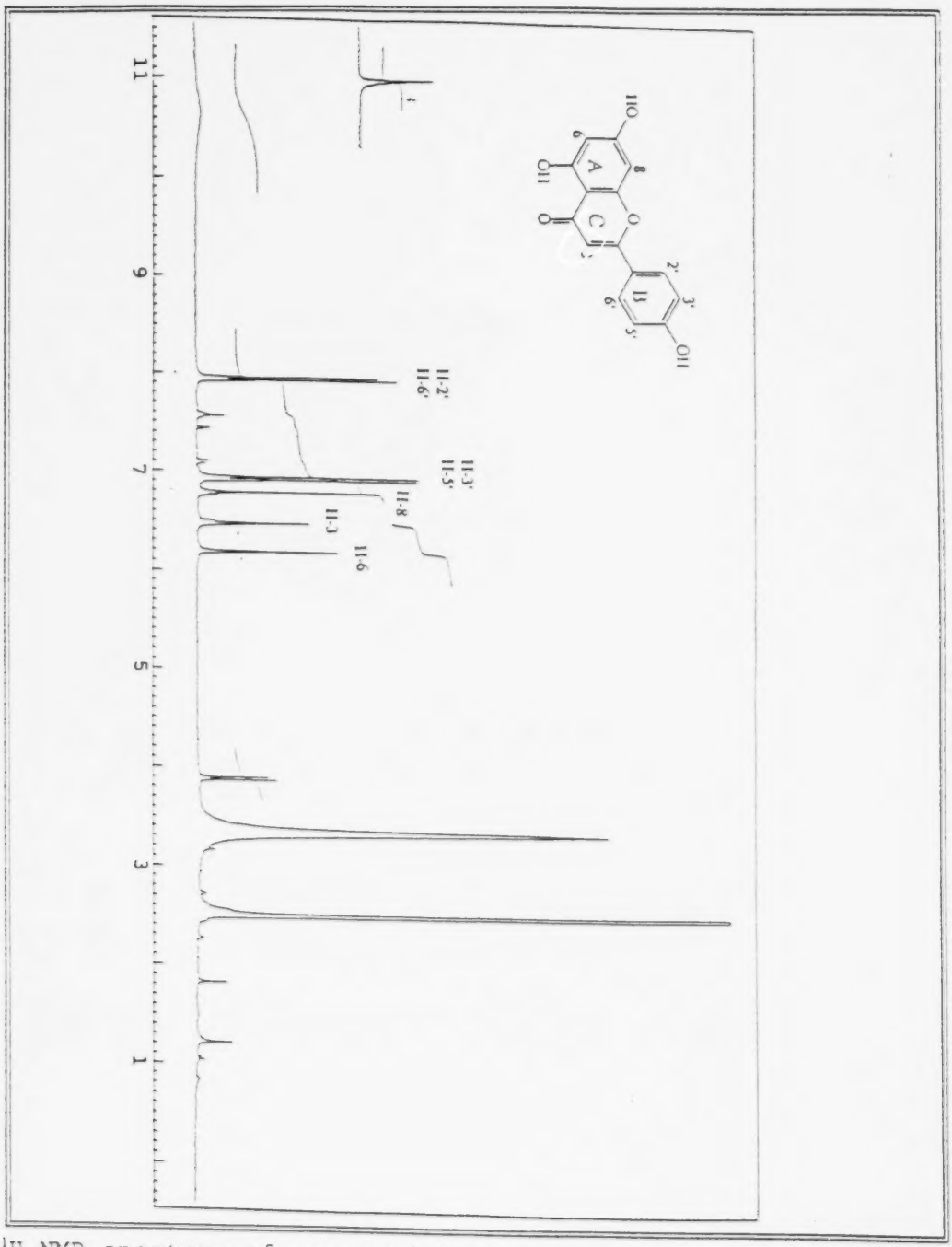


Mass spectrum of compound # 1

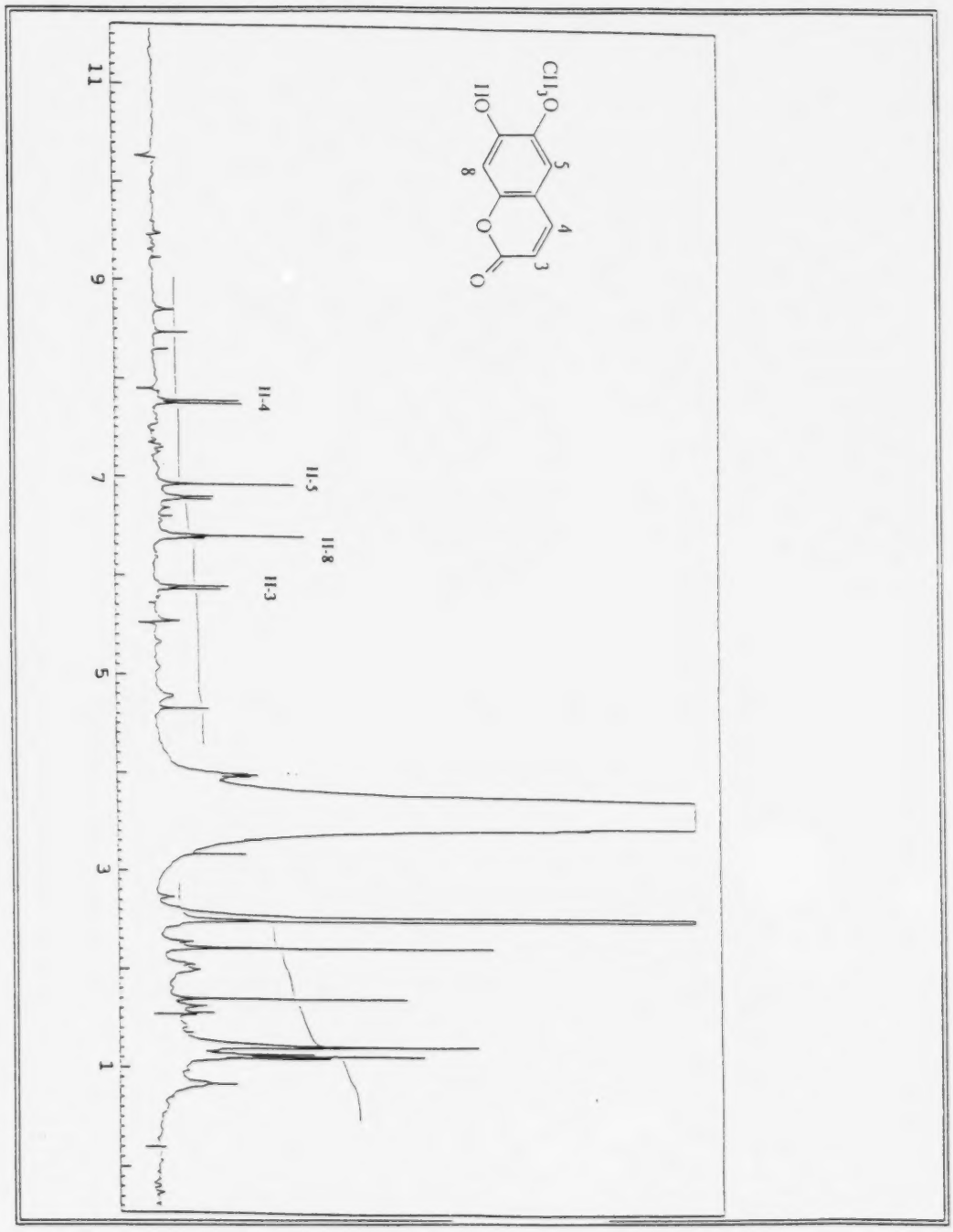


Mass spectrum of compound # 5

APPENDIX III. ^{1}H NMR of the isolated compounds



^{1}H NMR spectrum of compound # 4



^{1}H NMR spectrum of compound # 3